(FILE 'HOME' ENTERED AT 11:33:08 ON 25 SEP 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS' ENTERED AT 11:33:38 ON 25 SEP 2002

L1 536618 S DOMAIN
L2 107997 S SITE AND MUTAGENESIS
L3 815133 S ARGININE OR PHENYLALANINE OR TYROSINE OR TRYPTOPHAN OR TRYPTO
L4 259619 S ARG OR PHE OR TYR OR TRP OR LEU OR ISOLEU OR ISL
L5 6696 S L1 AND L2 AND (L3 OR L4)
L6 1628586 S AB OR ANTIBODY OR MAB OR MOAB OR IMMUNOADHESIN OR ADHESIN OR
L7 404 S L5 AND L6
L8 560757 S MULTIMERIZ? OR INTERFACE OR PROTUBERANCE OR CAVITY
L9 31 S L7 AND L8
L10 15 DUP REM L9 (16 DUPLICATES REMOVED)
L11 344753 S ASSEMBLY OR ASSEMBL?
L12 1366059 S CHAIN
L13 5 S L1 AND L11 AND L12 AND (L3 OR L4) AND L6 AND L8
L14 2 DUP REM L13 (3 DUPLICATES REMOVED)
L15 42 S L1 AND L11 AND L12 AND (L3 OR L4) AND L6
L16 25 DUP REM L15 (17 DUPLICATES REMOVED)
L17 78590 S HEAVY AND LIGHT
L18 30 S L1 AND L11 AND L12 AND (L3 OR L4) AND L17
L19 17 DUP REM L18 (13 DUPLICATES REMOVED)
L20 612589 S RECOMBINANT
L21 89619 S L20 AND L6
L22 2468 S L21 AND L17
L23 41 S L22 AND L8
L24 22 DUP REM L23 (19 DUPLICATES REMOVED)
L25 6874 S BISPECIFIC OR MULTISPECIFIC
L26 602 S L20 AND L6 AND L25
L27 36 S L11 AND L26
L28 14 DUP REM L27 (22 DUPLICATES REMOVED)
L29 1 S HETERDIMERIZ?
L30 5798 S HETERODIMERIZ?
L31 7 S L30 AND L3 AND L25
L32 3 DUP REM L31 (4 DUPLICATES REMOVED)
L33 1047220 S GLYCINE OR ALANINE OR SERINE OR THREONINE OR VALINE OR CYSTEI
L34 2 S L30 AND L33 AND L25
L35 2 DUP REM L34 (0 DUPLICATES REMOVED)
L36 605 S L30 AND L33
L37 269666 S ARG OR PHE OR TYR OR TRP OR LEU OR ILE
L38 1170 S (L3 OR L4 OR L33) AND L30
L39 0 S L38 AND L20 AND L17 AND L11
L40 172 S L38 AND L20
L41 0 S L40 AND L17
L42 6 S L40 AND L11
L43 6 DUP REM L42 (0 DUPLICATES REMOVED)

=> s l6 and l20 and l30

L44 71 L6 AND L20 AND L30

=> dup rem l44

PROCESSING COMPLETED FOR L44
L45 41 DUP REM L44 (30 DUPLICATES REMOVED)

=> s l10 or l16 or l19 or l24 or l28 or l32 or l35 or l43 or l45

L46 133 L10 OR L16 OR L19 OR L24 OR L28 OR L32 OR L35 OR L43 OR
L45

=> dup rem l46

PROCESSING COMPLETED FOR L46
L47 132 DUP REM L46 (1 DUPLICATE REMOVED)

SOURCE: THROMBOSIS AND HAEMOSTASIS, (2001 Dec) 86 (6) 1425-34.
Journal code: 7608063. ISSN: 0340-6245.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: , Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020713
Entered Medline: 20020712

AB We have investigated the effect of a new Leu196Pro mutation, identified in the MIDAS-like domain of the beta3 integrin subunit in a patient with type II Glanzmann thrombasthenia, on beta3 integrin receptor function. Expression of the mutant beta3Pro196 subunit in CHO cells, either associated with ***recombinant*** human alphaIIb or alphav, resulted in normal biosynthesis of beta3 and ***heterodimerization*** with alphav or alphaIIb, but selectively interfered with alphaIIbbeta3 maturation and transport to the cell surface. Functional analysis of the beta3 mutant receptors revealed strong inhibition of alphavbeta3-mediated cell spreading on immobilized fibrinogen, focal contact formation, p125FAK phosphorylation and fibrin clot retraction, as opposed to normal alphaIIbbeta3-mediated cell interaction with immobilized fibrinogen, focal contact translocation and signaling. In contrast, ***antibody*** - or DTT-activated mutant aIIbbeta3 was unable to bind soluble fibrinogen or the ligand mimetic PAC-1 monoclonal ***antibody*** , but underwent a conformational change following RGD peptide binding as demonstrated by AP5-LIBS epitope expression. These results suggest that (1) the highly conserved TL196T motif in the beta3 integrin subunit is located in a domain structurally important for the exposure of a functional binding site for soluble fibrinogen; and (2) that the MIDAS-like contact site in beta3 is not involved in alphaIIbbeta3-mediated cell adhesion to immobilized fibrinogen, while it is essential for alphavbeta3-mediated interaction with this ligand.

L47 ANSWER 20 OF 132 MEDLINE
ACCESSION NUMBER: 2001636114 MEDLINE
DOCUMENT NUMBER: 21543696 PubMed ID: 11688717
TITLE: Duocalins: engineered ligand-binding proteins with dual specificity derived from the lipocalin fold.
AUTHOR: Schlehuber S; Skerra A
CORPORATE SOURCE: Lehrstuhl fur Biologische Chemie, Technische Universitat Munchen, Freising-Weihenstephan, Germany.
SOURCE: BIOLOGICAL CHEMISTRY, (2001 Sep) 382 (9) 1335-42.
Journal code: 9700112. ISSN: 1431-6730.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20011105
Last Updated on STN: 20020320
Entered Medline: 20020319

AB Anticalins comprise a novel class of receptor proteins with predetermined ligand specificities which were engineered using the lipocalin fold. Attractive features of these artificial ligand-binding proteins include their small size and monomeric nature, being composed of a single polypeptide chain. Here we report the construction of a functional fusion protein from two independent anticalins, a so-called duocalin. The gene for the fusion protein was ***assembled*** from nucleotide sequences encoding an anticalin with fluorescein specificity on the one hand and an anticalin with digoxigenin specificity on the other. Both engineered lipocalins were previously selected from a random library prepared on the basis of the bilin-binding protein, a natural lipocalin abundant in insects. The corresponding fusion protein was expressed in a secretable form in E. coli cells and isolated from the periplasmic fraction using the Strep-tag method. The major fraction of the purified protein appeared to possess the proper pattern of altogether four disulphide bonds. The

TITLE: Anti-HLA-DR/anti-DOTA diabody construction in a modular gene design platform: ***bispecific*** antibodies for pretargeted radioimmunotherapy.
AUTHOR: DeNardo D G; Xiong C Y; Shi X B; DeNardo G L; DeNardo S J
CORPORATE SOURCE: Department of Internal Medicine, Section of Radiodiagnosis and Therapy, University of California Davis Medical Center, 1508 Alhambra Blvd, Suite 3100, Sacramento, CA 95816, USA.
CONTRACT NUMBER: CA-47829 (NCI)
SOURCE: CANCER BIOTHERAPY & RADIOPHARMACEUTICALS, (2001 Dec) 16 (6) 525-35.
Journal code: 9605408. ISSN: 1084-9785.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020703
Entered Medline: 20020702
AB ***Recombinant*** immunoglobulin libraries of single chain molecules (sc) from the variable domains of ***antibody*** light and heavy chains (Fv), have great promise for new approaches to radioimmunotherapy (RIT). However, creating and evaluating scFv from diverse sources is time consuming and differences in molecular format can influence in vitro and in vivo characteristics. Furthermore, scFv do not have optimal characteristics for targeting therapy to tumor because of their small size and univalent binding. Diabody molecules at least twice the size of scFv are better for RIT because bivalent and ***bispecific*** molecules can be constructed. A polymerase chain reaction (PCR) based primer system was created to easily convert scFv genes into a diabody gene format, once they have been placed into pCANTAB 5E, a readily available vector. The primer system for this diabody gene platform was developed and tested by constructing an anti-lymphoma/anti-chelate, ***bispecific*** diabody (anti-HLA-DR/anti-DOTA). Two mouse scFv libraries were screened for reactive clones using ***recombinant*** phage display techniques. Selected mouse anti-HLA-DR and anti-DOTA scFv genes were combined, ligated into the pCANTAB 5E vector that co-expressed these self- ***assembling*** scFv in E. coli as two mismatched nonlinked pairs (VHA-link-VLB; VHB-link-VLA). The diabody protein that was purified from periplasm had the expected molecular characteristics when analyzed by sequencing, chromatography, electrophoresis and Western blot. This modular gene design platform provides methodology for easy and rapid creation of diabody molecules from diverse scFv libraries. Diabodies from various scFv can easily be produced, thereby facilitating comparative preclinical studies en route to development of new tumor targeting molecules.

L47 ANSWER 23 OF 132 MEDLINE
ACCESSION NUMBER: 2001498225 MEDLINE
DOCUMENT NUMBER: 21431825 PubMed ID: 11545598
TITLE: Helix-stabilized Fv (hsFv) ***antibody*** fragments: substituting the constant domains of a Fab fragment for a heterodimeric coiled-coil domain.
AUTHOR: Arndt K M; Muller K M; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (2001 Sep 7) 312 (1) 221-8.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010910
Last Updated on STN: 20011022
Entered Medline: 20011018
AB ***Antibody*** Fv fragments would in principle be useful for a variety of biotechnological applications because of their small size and the

possibility to produce them in relatively large amounts in ***recombinant*** form; however, their limited stability is a drawback. To solve this problem, both domains are usually fused via a peptide linker to form a single-chain Fv (scFv) fragment, but in some cases this leads to a dimerization. We present an alternative format for stabilizing ***antibody*** Fv fragments. The C(H)1 and C(L) domain of the Fab fragment were replaced with a heterodimeric coiled coil (WinZip-A2B1), which had previously been selected using a protein-fragment complementation assay in Escherichia coli. This new ***antibody*** format was termed helix-stabilized Fv fragment (hsFv), and was compared to the corresponding Fv, Fab and single-chain Fv format. Bacterial growth and expression of the hsFv was significantly improved compared to the Fab fragment. The hsFv fragment formed a heterodimer of ***heavy*** and ***light*** chain with the expected molecular mass, also under conditions where the scFv fragment was predominantly dimeric. The hsFv fragment was significantly more stable than the Fv fragment, and nearly as stable as the scFv fragment under the conditions used (80 nM protein concentration). Thus, the format of a helix-stabilized Fv (hsFv) fragment can be a useful alternative to existing ***recombinant*** ***antibody*** formats, especially in cases where poor expression of Fab fragments or ***multimerization*** of scFv fragments is a problem.

L47 ANSWER 24 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:475568 CAPLUS
DOCUMENT NUMBER: 135:44981
TITLE: New ***recombinant*** bi- and trispecific ***antibody*** derivatives
AUTHOR(S): Mertens, Nico; Schoonjans, Reinilde; Willems, An; Schoonooghe, Steve; Leoen, Jannick; Grooten, Johan
CORPORATE SOURCE: Molecular Immunology Unit, Department of Molecular Biology, Flanders Interuniversity Institute of Biotechnology (VIB), Ghent University, Ghent, B-9000, Belg.
SOURCE: Focus on Biotechnology (2001), 1(Novel Frontiers in the Production of Compounds for Biomedical Use), 195-208
CODEN: FBOIAM
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bispecific antibodies (BsAb) are promising therapeutic tools in tomorrow's medicine. When constructing BsAbs, the final mol. size should be large enough to avoid rapid renal clearing, but small enough to allow efficient tissue distribution. In order to produce such intermediate sized BsAb, a good ***heterodimerization*** technique will improve existing prodn. methods. When considering ***recombinant*** expression of BsAbs, the ***heterodimerization*** motif can be incorporated into the mol. ***Recombinant*** BsAb can e.g. be made by fusing single chain variable fragments (scFv) to a ***heterodimerization*** domain. We compared the efficiency of the isolated CL and CH1 const. domains with complete Fab chains to drive ***heterodimerization*** of BsAbs in mammalian cells. We found that the isolated CL:CH1 domain interaction was inefficient for secretion of heterodimers. However, when the complete Fab chains were used, secretion of a ***heterodimerized*** bispecific ***antibody*** was successful. By C-terminal fusion of scFv mols. to the Fd- and the L-chains efficient ***heterodimerization*** in mammalian cells was obtained and a novel intermediate sized, disulfide stabilized BsAb could be efficiently produced. Since the Fab chain encodes a binding specificity on its own, bispecific (BsAb) or trispecific (TsAb) antibodies can be made. This gave rise to disulfide stabilized Fab-scFv BsAb (Bibody) or Fab-(scFv)2 TsAb (Tribody) of intermediate mol. size. ***Heterodimerization*** of the L and Fd-contg. fusion proteins was very efficient, and up to 90% of all secreted ***antibody*** fragments was in the desired ***heterodimerized*** format. All building blocks remained functional in the fusion product, and the bispecific character of the mols. as well as the functionality was demonstrated. Due to the high

heterodimerization efficiency, the ease of purifn. of the desired product from byproducts and the lack of post-prodn. processing, this method for producing bi- or trispecific antibodies in mammalian cells could become a method of choice for the prodn. of intermediate sized trispecific antibodies, BsAb with monovalent or bivalent binding for one antigen, or immunoconjugates.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L47 ANSWER 25 OF 132 MEDLINE
ACCESSION NUMBER: 2001396164 MEDLINE
DOCUMENT NUMBER: 21234909 PubMed ID: 11337278
TITLE: A new model for intermediate molecular weight ***recombinant*** bispecific and trispecific antibodies by efficient ***heterodimerization*** of single chain variable domains through fusion to a Fab-chain.
AUTHOR: Schoonjans R; Willems A; Schoonooghe S; Leoen J; Grooten J; Mertens N
CORPORATE SOURCE: Department of Molecular Biology, Molecular Immunology Unit, Flanders Interuniversity, Institute for Biotechnology (VIB), University of Ghent, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium.
SOURCE: BIOMOLECULAR ENGINEERING, (2001 Jun) 17 (6) 193-202. Journal code: 100928062. ISSN: 1389-0344.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010716
Last Updated on STN: 20010716
Entered Medline: 20010712

AB Due to their specificity and versatility in use, bispecific antibodies (BsAbs) are promising therapeutic tools in tomorrow's medicine, provided sufficient BsAb can be produced. Expression systems favoring efficient ***heterodimerization*** of intermediate-sized bispecific antibodies will significantly improve existing production methods. ***Recombinant*** BsAb can be made by fusing single chain variable fragments (scFv) to a ***heterodimerization*** domain. We compare the efficiency of the isolated CL and CH1 constant domains with complete Fab chains to drive ***heterodimerization*** of BsAbs in mammalian cells. We found that the isolated CL:CH1 domain interaction was inefficient for secretion of heterodimers. However, when the complete Fab chains were used, secretion of a ***heterodimerized*** bispecific ***antibody*** was successful. Since the Fab chain encodes a binding specificity on its own, bispecific (BsAb) or trispecific (TsAb) antibodies can be made by C-terminal fusion of scFv molecules to the L or Fd Fab chains. This gave rise to disulphide stabilized Fab-scFv BsAb (Bibody)or Fab-(scFv)2 TsAb (Tribody) of intermediate molecular size. ***Heterodimerization*** of the L and Fd-containing fusion proteins was very efficient, and up to 90% of all secreted ***antibody*** fragments was in the desired ***heterodimerized*** format. All building blocks remained functional in the fusion product, and the bispecific character of the molecules as well as the immunological functionality was demonstrated.

L47 ANSWER 26 OF 132 MEDLINE
ACCESSION NUMBER: 2002187529 MEDLINE
DOCUMENT NUMBER: 21917178 PubMed ID: 11920243
TITLE: Selective inactivation of Von Willebrand factor binding to glycoprotein IIb/IIIa and to inhibitor monoclonal ***antibody*** 9 by ***site*** -directed ***mutagenesis*** .
AUTHOR: Jumilly A L; Veyradier A; Ribba A S; Meyer D; Girma J P
CORPORATE SOURCE: INSERM U. 143 Hopital de Bicetre, Paris, France.
SOURCE: Hematol J, (2001) 2 (3) 180-7. Journal code: 100965523. ISSN: 1466-4860.
PUB. COUNTRY: England: United Kingdom

TITLE: BIBX1382BS, but not AG1478 or PD153035, inhibits the ErbB kinases at different concentrations in intact cells.
AUTHOR: Egeblad M; Mortensen O H; van Kempen L C; Jaattela M
CORPORATE SOURCE: Apoptosis Laboratory, Institute of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2001 Feb 16) 281 (1) 25-31.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010410
Last Updated on STN: 20010410
Entered Medline: 20010405

AB The activation of ErbB tyrosine kinase receptors (ErbB1, -2, -3, and -4) by ligand-induced homo- or ***heterodimerization*** regulates cell growth, death, and differentiation. AG1478 and PD153035 (also know as AG1517) have been adopted as specific ErbB1 inhibitors based on their high specificity for ErbB1 as compared to ErbB2 in in vitro kinase assays. We compared their ability to inhibit ErbB receptor signaling in intact cells to that of a novel ErbB receptor kinase inhibitor, BIBX1382BS. Neither AG1478 nor PD153035 displayed any specificity for ErbB1-mediated signaling induced by transforming growth factor alpha (TGF-alpha) as compared to signaling initiated through the other ErbB kinases. In contrast, BIBX1382BS was more potent at inhibiting signaling induced by TGF-alpha than that induced by neuregulin1-beta1 or anti-ErbB2 agonist antibodies. Interestingly, this compound blocked ***antibody*** -induced ErbB4 homodimer activation at even lower concentrations than ErbB1-triggered signaling. Thus, BIBX1382BS, but not AG1478 and PD153035, can be employed to differentiate between the ErbB kinases in intact cells when used at appropriate concentrations.

L47 ANSWER 29 OF 132 MEDLINE
ACCESSION NUMBER: 2001316759 MEDLINE
DOCUMENT NUMBER: 21283404 PubMed ID: 11388794
TITLE: Expression and purification of monospecific and ***bispecific*** ***recombinant*** ***antibody*** fragments derived from antibodies that block the CD80/CD86-CD28 costimulatory pathway.
AUTHOR: Dincq S; Bosman F; Buyse M A; Degrieck R; Celis L; de Boer M; Van Doorsselaere V; Sablon E
CORPORATE SOURCE: Department of Microbiology, Innogenetics NV, Industriepark Zwijnaarde 7, Box 4, B-9052 Gent, Belgium..
stephanie_dincq@innogenetics.be
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (2001 Jun) 22 (1) 11-24.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010827
Last Updated on STN: 20010827
Entered Medline: 20010823

AB The development of ***recombinant*** techniques for rapid cloning, expression, and characterization of cDNAs encoding ***antibody*** (***Ab***) subunits has revolutionized the field of ***antibody*** engineering. By fusion to heterologous protein domains, chain shuffling, or inclusion of self- ***assembly*** motifs, novel molecules such as ***bispecific*** Abs can be generated that possess the subset of functional properties designed to fit the intended application. We describe the engineering of ***Ab*** fragments produced in bacteria for blocking the CD28-CD80/CD86 costimulatory interaction in order to induce tolerance against transplanted organs. We designed single-chain Fv

FILE SEGMENT: 028 Urology and Nephrology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The full-length cDNA encoding the entire open reading frame (ORF) of rat myotubularin (rMTM) was isolated from a rat testis expression library by PCR. Among the three .apprx.2.9-kb cDNAs that were sequenced, one clone was different from the other two clones. It contained seven extra amino acids of FVVLNLQ; this short stretch of extra sequence was found between Gin421 and Phe422 within the SET (Suvar3-9, Enhancer-of-zeste, Trithorax) interacting ***domain*** (SID) of rMTM. The rMTM ORF had 1,713 bp encoding for a 571 amino acid polypeptide and a calculated molecular weight of 65.8 kDa. A comparison between its deduced amino acid sequence and the GenBank database using BLAST revealed a 53.1% identity with human myotubularin protein (hMTM1), which is a member of the protein ***tyrosine*** phosphatase (PTP) family associated with X-linked myotubular myopathy. A 22 amino acid peptide NH2-TKVNERYELCDTYPALLAVPAN was synthesized based on the deduced amino acid sequence of rMTM and used for ***antibody*** production. By using immunoblot analysis, a 66-kDa protein was indeed detected in both Sertoli and germ-cell cytosols. rMTM mRNA was found in various tissues but was predominantly expressed in the testis, ovary, and skeletal muscle. Sertoli cell rMTM expression was stimulated by germ cells and enhanced when inter-Sertoli junctions were being ***assembled*** in vitro. A drastic reduction in testicular rMTM steady-state mRNA level correlated with the depletion of germ cells from the testis in vivo following either glycerol or Ionidamine treatment. These results indicate that rMTM is a rat homologue of hMTM1 that may be a useful marker in monitoring the events of cell-cell interactions in the testis. (C) 2000 Wiley-Liss, Inc.

L47 ANSWER 43 OF 132 MEDLINE
ACCESSION NUMBER: 2001143113 MEDLINE
DOCUMENT NUMBER: 20556876 PubMed ID: 11105248
TITLE: Efficient ***heterodimerization*** of ***recombinant*** bi- and trispecific antibodies.
AUTHOR: Schoonjans R; Willems A; Grooten J; Mertens N
CORPORATE SOURCE: Department of Molecular Biology, University of Gent, Belgium.
SOURCE: BIOSEPARATION, (2000) 9 (3) 179-83.
Journal code: 9011423. ISSN: 0923-179X.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010308
AB Bispecific antibodies (BsAb) are promising therapeutic tools in tomorrow's medicine. Expression systems favoring efficient ***heterodimerization*** of intermediate-sized bispecific antibodies will significantly improve existing production methods. By C-terminal fusion of scFv molecules to the Fd- and the L-chains efficient ***heterodimerization*** in mammalian cells was obtained and a novel intermediate sized, disulfide stabilized BsAb could be efficiently produced. This type of ***antibody*** derivative easily allows for the production of trispecific antibodies, BsAb with bivalent binding for one antigen, or immunoconjugates.

L47 ANSWER 44 OF 132 MEDLINE
ACCESSION NUMBER: 2000137814 MEDLINE
DOCUMENT NUMBER: 20137814 PubMed ID: 10671300
TITLE: Soluble glycoprotein 130 (gp130) attenuates OSM- and LIF-induced cartilage proteoglycan catabolism.
AUTHOR: Hui W; Bell M; Carroll G
CORPORATE SOURCE: University Department of Medicine, University of Western Australia, Perth, Western Australia.
SOURCE: CYTOKINE, (2000 Feb) 12 (2) 151-5.

cancer cells was accompanied by increased tyrosine phosphorylation and ***heterodimerization*** of HER3 with HER2. In addition, we demonstrated that HER2 and HER3 receptors in colorectal cancer cells are constitutively phosphorylated on tyrosine residues and form heterodimeric complexes in the absence of exogenous NDF. Inhibition of HER2/HER3 signaling by an anti-HER3 ***mAb*** against the ligand binding site resulted in a decrease in the levels of constitutively activated HER2/ HER3 heterodimers, and the unexpected reduction of COX-2 expression. Activation of the HER2/HER3 pathway by NDF induced the activation of COX-2 promoter, expression of COX-2 mRNA, COX-2 protein and accumulation of prostaglandin E2 in the culture medium. Finally, we demonstrated that NDF promotes the ability of colorectal cancer cells to survive in an extracellular matrix milieu, such as Matrigel, and also to invade through a 8 microm porous membrane. These biological activities of NDF and its stimulation of cell proliferation are blocked by a specific inhibitor of COX-2. Taken together, our findings provide the first biochemical evidence of a possible role of the COX-2 pathway in the mitogenic action of NDF in colorectal cancer cells where it may be constitutively upregulated due to the autocrine/paracrine activation of HER2/ HER3 heterodimers.

L47 ANSWER 53 OF 132 MEDLINE
ACCESSION NUMBER: 1999336764 MEDLINE
DOCUMENT NUMBER: 99336764 PubMed ID: 10410983
TITLE: Intracellular and cell surface displayed single-chain diabodies.
AUTHOR: Kontermann R E; Muller R
CORPORATE SOURCE: Institut fur Molekularbiologie und Tumorforschung, Philipps-Universitat Marburg, Germany.. rek@imt.uni-marburg.de
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1999 Jun 24) 226 (1-2) 179-88.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990827
Last Updated on STN: 19990827
Entered Medline: 19990813

AB Intracellularly expressed ***antibody*** fragments have found various applications in therapy by virtue of their ability to inhibit the function of cellular proteins or interfere with subcellular trafficking. Bivalent ***antibody*** fragments might further improve this inhibitory potential by increasing the functional affinity and ***bispecific*** ***antibody*** fragments may also be useful for the intracellular retargeting of molecules. Here, we have evaluated the functional expression of intracellular diabodies. A previously constructed secreted ***bispecific*** single-chain diabody directed against carcinoembryonic antigen and Escherichia coli beta-galactosidase was modified for subcellular targeting to the cell surface membrane, endoplasmic reticulum, mitochondria, cytoplasm, and nucleus. Subcellular localisation was analysed by immunofluorescence, and the ***assembly*** of functional antibodies was analysed by binding of beta-galactosidase to the ***antibody*** fragment and subsequent substrate conversion. ***Bispecific*** single-chain diabodies could be directed to all subcellular compartments analysed. However, functional ***assembly*** was only observed for single-chain diabodies retained in the endoplasmic reticulum or displayed in the cell membrane while no antigen binding activity was seen with diabodies directed to the cytoplasm, nucleus, or mitochondria. The results demonstrate the functional expression of ***bispecific*** ***recombinant*** ***antibody*** fragments in the secretory pathway and integration into the plasma membrane of mammalian cells.

L47 ANSWER 54 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999095632 EMBASE

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE:. GENBANK-AF091121
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981216

AB Nuclear hormone receptors comprise a characteristic family of transcription factors found in vertebrates, insects and nematodes. Here we show by cDNA and gene cloning that a Cnidarian, Tripedalia cystophora, possesses a retinoid receptor (jRXR) with remarkable homology to vertebrate retinoic acid X receptors (RXRs). Like vertebrate RXRs, jRXR binds 9-cis retinoic acid (Kd = 4 x 10(-10) M) and binds to the DNA sequence, PuGGTCA as a monomer in vitro. jRXR also ***heterodimerizes*** with Xenopus TR beta on a thyroid responsive element of a direct repeat separated by 4 bp. A jRXR binding half-site capable of interacting with (His6)jRXR fusion protein was identified in the promoters of three T. cystophora crystallin genes that are expressed highly in the eye lens of this jellyfish. Because crystallin gene expression is regulated by retionoid signaling in vertebrates, the jellyfish crystallin genes are candidate in vivo targets for jRXR. Finally, an ***antibody*** prepared against (His6)jRXR showed that full-length jRXR is expressed at all developmental stages of T. cystophora except the ephydra, where a smaller form replaces is. These data show that Cnidaria, a diploblastic phylum ancestral to the triploblastic invertebrate and subsequent vertebrate lineages, already have an RXR suggesting that RXR is an early component of the regulatory mechanisms of metazoa.

L47 ANSWER 59 OF 132 MEDLINE
ACCESSION NUMBER: 1998409447 MEDLINE
DOCUMENT NUMBER: 98409447 PubMed ID: 9737871
TITLE: Factors influencing the dimer to monomer transition of an ***antibody*** single-chain Fv fragment.
AUTHOR: Arndt K M; Muller K M; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: BIOCHEMISTRY, (1998 Sep 15) 37 (37) 12918-26.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981029
Last Updated on STN: 19981029
Entered Medline: 19981020

AB ***Antibody*** single-chain Fv (scFv) fragments are able to form dimers under certain conditions, and the extent of dimerization appears to depend on linker length, ***antibody*** sequence, and external factors. We analyzed the factors influencing dimer-monomer equilibrium as well as the rate of interconversion, using the scFv McPC603 as a model system. In this molecule, the stability of the VH-VL interaction can be conveniently varied by adjusting the ionic strength (because of its influence on the hydrophobic effect), by pH (presumably because of the presence of titratable groups in the ***interface***), and by the presence or absence of the antigen phosphorylcholine, which can be rapidly removed due to its very fast off-rate. It was found that the monomer is the thermodynamically stable form with linkers of 15 and 25 amino acids length under all conditions tested (35 &mgr;M or less). The dimer is initially formed in periplasmic expression, presumably by domain swapping, and can be trapped by all factors which stabilize the VH-VL ***interface*** , such as the presence of the antigen, high ionic strength, and pH below 7.5. Under all other conditions, it converts to the monomer. Predominantly monomer is obtained during in vitro folding. Monomer is stabilized against dimerization at very high concentrations by the same factors which stabilize the VH-VL interaction. These results should be helpful in producing molecules with defined oligomerization

(Glutathione S-transferase)-E2F-1 fusion protein as a probe. One of the clones encodes E2FBP1 which has the helix-loop-helix (HLH) motif, but lacks the basic domain and the zipper structure usually found at N- and C-terminal sides to the HLH motif, respectively. The arrangement of amino acids in the helix 1 and helix 2 regions is quite similar to those of Mxi and Mad, but different from those of E2F-1 and DP-1. Western blot analysis of the immunoprecipitates prepared with anti-E2FBP1 ***antibody*** showed that E2FBP1 associates with both E2F-1 and DP-1 in vivo. E2FBP1 alone has no DNA binding activity, but bind to the E2F site through ***heterodimerization*** with E2F-1 but not with DP-1. Although E2FBP1 lacks the transactivation domain, it stimulates E2F site-dependent transcription in cooperation with E2F-1.

L47 ANSWER 66 OF 132 MEDLINE
ACCESSION NUMBER: 1998343786 MEDLINE
DOCUMENT NUMBER: 98343786 PubMed ID: 9680192
TITLE: Reengineering immunoglobulin ***domain*** interactions by introduction of charged residues.
AUTHOR: Raffen R; Stevens P W; Boogaard C; Schiffer M; Stevens F J
CORPORATE SOURCE: Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, IL 60439, USA.
CONTRACT NUMBER: DK43757 (NIDDK)
GM16829 (NIGMS)
SOURCE: PROTEIN ENGINEERING, (1998 Apr) 11 (4) 303-9.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980922
AB The formation of the ***antibody*** variable ***domain*** binding unit (Fv) is the net result of three competing ***assembly*** reactions. The affinities of concurrent homologous interactions of ***heavy*** and ***light*** ***chain*** variable domains limits the heterologous interaction leading to productive formation of the Fv. To address the possible role of ***light*** ***chain*** dimerization in this phenomenon, the Gln38 residue at the dimer ***interface*** of an immunoglobulin ***light*** ***chain*** variable ***domain*** (VL) was replaced by charged amino acids. The effects of these mutations on VL homodimer formation were monitored by small-zone size exclusion HPLC and the affinities of interaction were determined by computer simulation. Reduced VL homodimerization was observed in three of the four mutants, Q38R, Q38D and Q38K. The association constants for the Q38R and Q38D homodimers were 1.2 x 10(4) and 3.2 x 10(3) M(-1), respectively. This corresponded to a 20-75-fold reduction in the homodimer association constant relative to the wild-type VL, which had an association constant of 2.4 x 10(5) M(-1). Surprisingly, the fourth charge mutant, Q38E, had a higher association constant than the wild-type VL. The potential for charged residues to facilitate heterodimeric ***assembly*** of immunoglobulin domains was also tested. Heterodimerization was observed between the Q38D and Q38R V(L)s, but with an association constant of 4.7 x 10(4) M(-1), approximately fivefold lower than that obtained for homodimerization of the native V(L). In addition, replacement of the neutral, solvent-accessible Gln38 residue with either Asp or ***Arg*** was found to be significantly destabilizing. These results suggest that charged residues could be introduced at immunoglobulin ***domain*** interfaces to guide heterodimer formation and to minimize unfavorable competing homologous associations. Nonetheless, these apparently simple modifications may also result in unintended consequences that are likely to depend upon structural features of particular variable domains.

L47 ANSWER 67 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:524202 CAPLUS
DOCUMENT NUMBER: 131:270696

TITLE: Critical role of conserved amino acid residues in complementarity determining regions for ***antibody*** specificity and polypeptide-***chain*** ***assembly***

AUTHOR(S): Kinoshita, Takeshi; Suzuki, Yasuhiko; Ida, Sohji; Naito, Akihiro; Wakamiya, Nobutaka; Kozono, Haruo; Azuma, Takachika

CORPORATE SOURCE: Central Research Laboratory, Nippon Suisan Ltd., Tokyo, 192, Japan

SOURCE: Research Communications in Biochemistry and Cell & Molecular Biology (1998), 2(3 & 4), 275-288
CODEN: RCBBFC; ISSN: 1087-111X

PUBLISHER: PJD Publications Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Fab fragments of a wild type or mutants of an anti-(4-hydroxy-3-nitrophenyl)acetyl ***mAb*** , B2, were expressed on the surface of a filamentous phage in order to examine the role of conserved amino acid residues at positions 32, 50, and 60 in complementarity detg. regions. These had been predicted previously as specificity-detg. residues. Phages expressing mutant Fabs with replacement of a single amino acid at these positions in complementarity detg. regions of the ***heavy*** - ***chain*** V region showed a large to complete loss of ability to bind haptens. In addn., substitution of Tyr60 hindered formation of Fab, suggesting that this amino acid residue is crit. for the interaction between V domains in ***heavy*** and ***light*** chains. Thus, the amino acid residues conserved in somatic mutation of complementarity detg. regions are important in detg. ***Ab*** -specificity as well as in inter-V ***domain*** interactions.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L47 ANSWER 68 OF 132 MEDLINE

ACCESSION NUMBER: 1998149664 MEDLINE

DOCUMENT NUMBER: 98149664 PubMed ID: 9490020

TITLE: The first constant domain (C(H)1 and C(L)) of an ***antibody*** used as ***heterodimerization*** domain for ***bispecific*** miniantibodies.

AUTHOR: Muller K M; Arndt K M; Strittmatter W; Pluckthun A

CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.

SOURCE: FEBS LETTERS, (1998 Jan 30) 422 (2) 259-64.
Journal code: 0155157. ISSN: 0014-5793.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199803

ENTRY DATE: Entered STN: 19980326
Last Updated on STN: 20000303
Entered Medline: 19980317

AB ***Bispecific*** miniantibodies were constructed by genetically fusing the C(H)1 domain of an IgG1 to the C-terminus of a single-chain Fv fragment (scFv-425), specific for the EGF receptor, and fusing the C(L) domain of a kappa light chain to the C-terminus of a scFv specific for CD2 (scFv-M1). An efficient dicistronic gene arrangement for functional expression in Escherichia coli was constructed. Immunoblots demonstrated correct domain ***assembly*** and the formation of the natural C(H)1-C(L) disulfide bridge. Gel filtration confirmed the correct size, sandwich ELISAs demonstrated ***bispecific*** functionality, and SPR biosensor measurements determined binding to EGF-R in comparison to bivalent constructs. ***Bispecific*** anti-EGF-R/anti-CD2 miniantibodies are candidates for the immunotherapy of cancer.

L47 ANSWER 69 OF 132 MEDLINE

ACCESSION NUMBER: 1999086155 MEDLINE

DOCUMENT NUMBER: 99086155 PubMed ID: 9870608

TITLE: Cell surface GPI-anchoring of CD45 isoforms.

PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D78609
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970407
Last Updated on STN: 19970407
Entered Medline: 19970326

AB Many plant basic leucine-zipper (bZIP) proteins have been isolated several of which have been shown to play a role in seed-specific gene expression. We isolated a novel bZIP protein (REB) gene encoding 425 amino acid residues from rice endosperm, which is similar to Opaque-2 ***heterodimerizing*** protein (OHP) of maize. The gene product, termed REB, contains Pro- and Gly-rich regions at its N terminus, followed by the typical basic and leucine-repeat regions. ***Recombinant*** REB binds to the region from -754 to -562 in the alpha-globulin gene promoter, but not to promoters of other major storage genes such as glutelin, prolamin and albumin. The 5' region of the alpha-globulin gene possesses three binding sites for REB, which were determined as GCCACGT(A/C)AG, by using synthetic oligonucleotides. A Super-shift assay using anti-REB ***antibody*** suggested that REB is a major DNA-binding protein for the alpha-globulin gene promoter in rice endosperm.

L47 ANSWER 77 OF 132 MEDLINE
ACCESSION NUMBER: 97337429 MEDLINE
DOCUMENT NUMBER: 97337429 PubMed ID: 9194169
TITLE: Disrupting the hydrophobic patches at the ***antibody*** variable/constant domain ***interface*** : improved in vivo folding and physical characterization of an engineered scFv fragment.
AUTHOR: Nieba L; Honegger A; Krebber C; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: PROTEIN ENGINEERING, (1997 Apr) 10 (4) 435-44.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970902
Last Updated on STN: 19970902
Entered Medline: 19970818

AB By constructing Fv and single-chain Fv (scFv) fragments of antibodies, the variable domains are taken out of their natural context in the Fab fragment, where they are associated with the constant domains of the ***light*** (CL) and ***heavy*** chain (CH1). As a consequence, all residues of the former variable/constant domain ***interface*** become solvent exposed. In an analysis of 30 non-redundant Fab structures it was found that at the former variable/constant domain ***interface*** of the Fv fragment the frequency of exposed hydrophobic residues is much higher than in the rest of the Fv fragment surface. We investigated the importance of these residues for different properties such as folding in vivo and in vitro, thermodynamic stability, solubility of the native protein and antigen affinity. The experimental model system was the scFv fragment of the anti-fluorescein ***antibody*** 4-4-20, of which only 2% is native when expressed in the periplasm of Escherichia coli. To improve its in vivo folding, a mutagenesis study of three newly exposed interfacial residues in various combinations was carried out. The replacement of one of the residues (V84D in VH) led to a 25-fold increase of the functional periplasmic expression yield of the scFv fragment of the ***antibody*** 4-4-20. With the purified scFv fragment it was shown that the thermodynamic stability and the antigen binding constant are not influenced by these mutations, but the rate of the thermally induced aggregation reaction is decreased. Only a minor effect on the solubility of the native protein was observed, demonstrating that the mutations prevent aggregation during folding and not of the native protein. Since

the construction of all scFv fragments leads to the exposure of these residues at the former variable/constant domain ***interface*** , this strategy should be generally applicable for improving the in vivo folding of scFv fragments and, by analogy, also the in vivo folding of other engineered protein domains.

L47 ANSWER 78 OF 132 MEDLINE
ACCESSION NUMBER: 1998145605 MEDLINE
DOCUMENT NUMBER: 98145605 PubMed ID: 9099794
TITLE: ***Antibody*** engineering.
AUTHOR: Hayden M S; Gilliland L K; Ledbetter J A
CORPORATE SOURCE: Department of Autoimmunity and Transplantation, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, Washington, WA 98121, USA..
Martha_S._Hayden@ccmail.bms.com
SOURCE: CURRENT OPINION IN IMMUNOLOGY, (1997 Apr) 9 (2) 201-12.
Ref: 127
Journal code: 8900118. ISSN: 0952-7915.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980422
Last Updated on STN: 19980422
Entered Medline: 19980415
AB The development of ***recombinant*** techniques for the rapid cloning, expression, and characterization of cDNAs encoding ***antibody*** (***Ab***) subunits has revolutionized the field of ***antibody*** engineering. By fusion to heterologous protein domains, chain shuffling, and inclusion of self- ***assembly*** motifs, novel molecules such as ***bispecific*** Abs can now be generated which possess the subset of functional properties designed to fit the intended application. Rapid technological developments in phage display of peptides and proteins have led to a plethora of applications directed towards immunology and ***antibody*** engineering. Many of the problems associated with the therapeutic use of Abs are being addressed by the application of these new techniques.

L47 ANSWER 79 OF 132 MEDLINE
ACCESSION NUMBER: 1998063340 MEDLINE
DOCUMENT NUMBER: 98063340 PubMed ID: 9398404
TITLE: IL-2 and IL-7 induce ***heterodimerization*** of STAT5 isoforms in human peripheral blood T lymphoblasts.
AUTHOR: Rosenthal L A; Winestock K D; Finbloom D S
CORPORATE SOURCE: Division of Cytokine Biology, Food and Drug Administration, Bethesda, Maryland 20892-4555, USA.
SOURCE: CELLULAR IMMUNOLOGY, (1997 Nov 1) 181 (2) 172-81.
Journal code: 1246405. ISSN: 0008-8749.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980122
Last Updated on STN: 19980122
Entered Medline: 19980108
AB Despite differences in T cell responses induced by interleukin (IL)-2 and IL-7, both cytokines modulate T cell functions by activation of signal transducers and activators of transcription (STAT) proteins. We examined the contribution of the two isoforms of STAT5, STAT5A and STAT5B, to IL-2- and IL-7-induced activation of human peripheral blood T lymphoblasts. Both cytokines induced ***assembly*** of STAT5A and STAT5B containing complexes capable of binding to the interferon-gamma activation sequence (GAS), and these complexes rapidly translocated (within 1 min) into the

nucleus of IL-2- or IL-7-treated cells. The kinetics of this translocation were delayed in IL-7-treated as compared to IL-2-treated cells. IL-2 and IL-7 were equivalent in their ability to induce ***tyrosine*** phosphorylation of STAT5A and STAT5B and to facilitate binding of these STATs to an immobilized GAS element. Both IL-2 and IL-7 induced substantial amounts of STAT5A/STAT5B ***heterodimerization*** . Moreover, we observed constitutive association of STAT3 with each STAT5 isomer. These data suggest that IL-2 and IL-7 induce ***assembly*** of STAT heterodimers in a similar manner and that subsequent cellular responses may be driven by induction of similar sets of genes.

L47 ANSWER 80 OF 132 MEDLINE
ACCESSION NUMBER: 1998137001 MEDLINE
DOCUMENT NUMBER: 98137001 PubMed ID: 9476520
TITLE: Recognition of E. coli ***tryptophan*** synthase by single- ***chain*** Fv fragments: comparison of PCR-cloning variants with the parental antibodies.
AUTHOR: Bregegere F; England P; Djavadi-Ohaniance L; Bedouelle H
CORPORATE SOURCE: Unite de Biochimie Cellulaire (CNRS URA 1129), Institut Pasteur, Paris, France.
SOURCE: JOURNAL OF MOLECULAR RECOGNITION, (1997 Jul-Aug) 10 (4) 169-81.
Journal code: 9004580. ISSN: 0952-3499.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980312
Last Updated on STN: 19980312
Entered Medline: 19980303
AB The use of a recombinant ***antibody*** fragment instead of a complete ***antibody*** , as a conformational probe for protein structure and folding studies, can be technically advantageous provided that the recombinant fragment and its parental ***antibody*** recognize the antigen through the same mechanism. Monoclonal antibodies mAb19 and mAb93 are directed against the TrpB2 subunit of Escherichia coli ***tryptophan*** synthase and they have been extensively used as conformational probes of this protein. DNA sequences coding for single- ***chain*** variable fragments (scFv) of mAb19 and mAb93 were cloned and ***assembled*** by reverse transcription of the mRNAs from hybridomas and PCR amplification. A specialized plasmid vector, pFBX, was constructed; it enabled to express the scFvs as hybrids with the maltose-binding protein (MalE) in E. coli, and to purify them by affinity chromatography on cross-linked amylose. Six independent clones were sequenced for each hybridoma. All of them had differences in their nucleotide and amino acid sequences. A competition ELISA and the BIAcore biosensor apparatus were used to compare the energetics and kinetics with which the parental antibodies and the hybrids bound TrpB2. The antigen binding properties of the hybrids were close to those of the parental antibodies and they were only weakly affected by the differences of sequence between the clones, with one exception. The stability of one of the hybrids and its antigen binding properties were strongly modified by a change of Gln6 into Glu, introduced into its VH ***domain*** by the PCR primers. Simple models of bimolecular interaction did not fully account for the kinetic profiles obtained with the parental antibodies and the hybrids, and this complexity suggested the existence of a conformational heterogeneity in these molecules.

L47 ANSWER 81 OF 132 MEDLINE
ACCESSION NUMBER: 97380308 MEDLINE
DOCUMENT NUMBER: 97380308 PubMed ID: 9237098
TITLE: Enzyme immunoassays using ***bispecific*** diabodies.
AUTHOR: Kontermann R E; Martineau P; Cummings C E; Karpas A; Allen D; Derbyshire E; Winter G
CORPORATE SOURCE: MRC Centre for Protein Engineering, Cambridge, UK.
SOURCE: IMMUNOTECHNOLOGY, (1997 Jun) 3 (2) 137-44.

Journal code: 9511979. ISSN: 1380-2933.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970916
Last Updated on STN: 19970916
Entered Medline: 19970904

AB BACKGROUND: ***Bispecific*** antibodies with a first binding specificity to a target antigen and a second to an enzyme have great potential in enzyme immunoassays. As ***bispecific*** antibodies are difficult to make, the use of ***recombinant*** ***bispecific*** ***antibody*** fragments may provide a breakthrough. OBJECTIVES: To make ***bispecific*** ***antibody*** fragments directed against an enzyme and to demonstrate their application in enzyme immunoassays. STUDY DESIGN: ***Bispecific*** ***antibody*** fragments were ***assembled*** as diabodies (Holliger P., Prospero T., Winter G. Proc. Natl. Acad. Sci. USA 90, 1993, 6444-6448) directed to an enzyme, E. coli beta-galactosidase, and to each of three target antigens, hen-egg lysozyme (HEL), carcinoembryonic antigen (CEA), and HIV gpl20 (HIV). The diabodies were then evaluated in immunoassays. RESULTS: The HEL diabody was shown to recruit beta-galactosidase in a microtiter plate immunoassay in which diabody and enzyme were co-incubated with antigen, washed and enzyme substrate added. The CEA diabody was shown to detect CEA by immunocytochemical staining of transfected, CEA-expressing HeLa cells and of adenocarcinoma colon tissue sections, and the HIV diabody to detect gpl20 in immunoblots of total cell extracts. CONCLUSION: The results illustrate the diagnostic potential of diabodies in enzyme immunoassays.

L47 ANSWER 82 OF 132 MEDLINE
ACCESSION NUMBER: 97184607 MEDLINE
DOCUMENT NUMBER: 97184607 PubMed ID: 9032408
TITLE: Reliable cloning of functional ***antibody*** variable domains from hybridomas and spleen cell repertoires employing a reengineered phage display system.
AUTHOR: Krebber A; Bornhauser S; Burmester J; Honegger A; Willuda J; Bosshard H R; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut der Universitat Zurich, Switzerland.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1997 Feb 14) 201 (1) 35-55.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X99506; GENBANK-X99507; GENBANK-X99508; GENBANK-X99509
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970321
Last Updated on STN: 19990129
Entered Medline: 19970313

AB A prerequisite for the use of ***recombinant*** ***antibody*** technologies starting from hybridomas or immune repertoires is the reliable cloning of functional immunoglobulin genes. For this purpose, a standard phage display system was optimized for robustness, vector stability, tight control of scFv-delta geneIII expression, primer usage for PCR amplification of variable region genes, scFv assembly strategy and subsequent directional cloning using a single rare cutting restriction enzyme. This integrated cloning, screening and selection system allowed us to rapidly obtain antigen binding scFvs derived from spleen-cell repertoires of mice immunized with ampicillin as well as from all hybridoma cell lines tested to date. As representative examples, cloning of monoclonal antibodies against a his tag, leucine zippers, the tumor marker EGP-2 and the insecticide DDT is presented. Several hybridomas whose genes could not be cloned in previous experimental setups, but were successfully obtained with the present system, expressed high amounts of

aberrant ***heavy*** and ***light*** chain mRNAs, which were amplified by PCR and greatly exceeded the amount of binding ***antibody*** sequences. These contaminating variable region genes were successfully eliminated by employing the optimized phage display system, thus avoiding time consuming sequencing of non-binding scFv genes. To maximize soluble expression of functional scFvs subsequent to cloning, a compatible vector series to simplify modification, detection, ***multimerization*** and rapid purification of ***recombinant*** ***antibody*** fragments was constructed.

L47 ANSWER 83 OF 132 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 97375581 MEDLINE
DOCUMENT NUMBER: 97375581 PubMed ID: 9231898
TITLE: Stable heterodimers from remodeling the domain interface of a homodimer using a phage display library.
AUTHOR: Atwell S; Ridgway J B; Wells J A; Carter P
CORPORATE SOURCE: Department of Protein Engineering, Genentech Inc., South San Francisco, CA 94080, USA.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1997 Jul 4) 270 (1) 26-35.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970902
Last Updated on STN: 19980206
Entered Medline: 19970815
AB Structure-guided phage display was used to select for combinations of interface residues for ***antibody*** C(H)3 domains that promote the formation of stable heterodimers. A C(H)3 "knob" mutant was made by replacement of a small residue, threonine, with a larger one, tryptophan: T366W. A library of C(H)3 "hole" mutants was then created by randomizing residues 366, 368 and 407, which are in proximity to the knob on the partner C(H)3 domain. The C(H)3 knob mutant was fused to a peptide flag and the C(H)3 hole library was fused to M13 gene III. Phage displaying stable C(H)3 heterodimers were recovered by panning using an anti-flag ***antibody*** . Phage-selected C(H)3 heterodimers differed in sequence from the previously designed heterodimer T366W-Y407'A, and most clones tested were more stable to guanidine hydrochloride denaturation. The thermal stability of individual C(H)3 domains secreted from Escherichia coli was analyzed by differential scanning calorimetry. One heterodimer, T366W-T366'S:L368'A:Y407'V, had a t(m) of 69.4 degrees C, which is 4.0 deg.C higher than that for the designed heterodimer and 11.0 deg.C lower than that for the wild-type homodimer. The phage-selected C(H)3 mutant maintained the preference for forming heterodimers over homodimers as judged by near-quantitative formation of an ***antibody*** / ***immunoadhesin*** hybrid in a cotransfection assay. Phage optimization provides a complementary and more comprehensive strategy to rational design for engineering homodimers for ***heterodimerization*** .

L47 ANSWER 84 OF 132 MEDLINE
ACCESSION NUMBER: 96411744 MEDLINE
DOCUMENT NUMBER: 96411744 PubMed ID: 8810297
TITLE: Ca2+ binding to the first epidermal growth factor-like ***domain*** of human blood coagulation factor IX promotes enzyme activity and factor VIII ***light*** ***chain*** binding.
AUTHOR: Lenting P J; Christophe O D; Maat H; Rees D J; Mertens K
CORPORATE SOURCE: Department of Plasma Protein Technology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, 1066 CX Amsterdam, the Netherlands.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Oct 11) 271 (41) 25332-7.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

binding sites. Finally, we looked more closely at the C-terminal binding region of the alpha3(IV) NC1 ***domain*** . Since the lysines in that region have been previously advanced as possible contact sites, we created several substitutions within the C-terminal epitope of the alpha3 NC1 ***domain*** . Substitution of lysines to alanines revealed lysines 219 and 229 as essential for ***antibody*** binding to this distal site; no lysines were present in the NC1 part of the helix-NC1 junction region. Substitutions involving ***arginine*** and cysteines to alanines in the same C-terminal region did not produce significant reductions in ***antibody*** binding. In summary, our findings characterize two Goodpasture epitopes confined to each end of the alpha3 NC1 ***domain*** ; one is lysine-dependent, and the other is not. We propose, as a hypothetical model, that these two immunologically privileged regions fold to form an optimal pathogenic structure within the NC1 ***domain*** of the alpha3 ***chain*** . These sites are subsequently concealed by NC1 hexamer ***assembly*** of type IV collagen.

L47 ANSWER 87 OF 132 MEDLINE
ACCESSION NUMBER: 96205948 MEDLINE
DOCUMENT NUMBER: 96205948 PubMed ID: 8631797
TITLE: An immunological approach reveals biological differences between the two NDF/heregulin receptors, ErbB-3 and ErbB-4.
AUTHOR: Chen X; Levkowitz G; Tzahar E; Karunagaran D; Lavi S; Ben-Baruch N; Leitner O; Ratzkin B J; Bacus S S; Yarden Y
CORPORATE SOURCE: Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Mar 29) 271 (13) 7620-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960715
Last Updated on STN: 20000303
Entered Medline: 19960703

AB The group of subtype I transmembrane tyrosine kinases includes the epidermal growth factor (EGF) receptor (ErbB-1), an orphan receptor (ErbB-2), and two receptors for the Neu differentiation factor (NDF/heregulin), namely: ErbB-3 and ErbB-4. Here we addressed the distinct functions of the two NDF receptors by using an immunological approach. Two sets of monoclonal antibodies (mAbs) to ErbB-3 and ErbB-4 were generated through immunization with ***recombinant*** ectodomains of the corresponding receptors that were fused to immunoglobulin. We found that the shared ligand binds to highly immunogenic, but immunologically distinct sites of ErbB-3 and ErbB-4. NDF receptors differed also in their kinase activities; whereas the catalytic activity of ErbB-4 was activable by mAbs, ErbB-3 underwent no activation by mAbs in living cells. Likewise, down-regulation of ErbB-4, but not ErbB-3, was induced by certain mAbs. By using the generated mAbs, we found that the major NDF receptor on mammary epithelial cells is a heterodimer of ErbB-3 with ErbB-2, whereas an ErbB-1/ErbB-2 heterodimer, or an ErbB-1 homodimer, is the predominant species that binds EGF. Consistent with ErbB-2 being a shared receptor subunit, its tyrosine phosphorylation was increased by both heterologous ligands and it mediated a trans-inhibitory effect of NDF on EGF binding. Last, we show that the effect of NDF on differentiation of breast tumor cells can be mimicked by anti-ErbB-4 antibodies, but not by mAbs to ErbB-3. Nevertheless, an ErbB-3-specific ***mAb*** partially inhibited the effect of NDF on cellular differentiation. These results suggest that homodimers of ErbB-4 are biologically active, but ***heterodimerization*** of the kinase-defective ErbB-3, probably with ErbB-2, is essential for transmission of NDF signals through ErbB-3.

L47 ANSWER 88 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97017382 EMBASE
DOCUMENT NUMBER: 1997017382

Entered Medline: 19950907

AB The nuclear vitamin D receptor (VDR) binds the 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) hormone with high affinity and elicits its actions to stimulate gene expression in target cells by binding to the vitamin D-responsive element (VDRE). VDREs in such positively controlled genes as osteocalcin, osteopontin, beta 3 integrin and vitamin D-24-OHase are direct hexanucleotide repeats with a spacer of three nucleotides. The present studies of VDR/VDRE interaction utilized full-length human vitamin D receptor (hVDR) that was overexpressed in E. coli, purified to near homogeneity (> 95%), and its authenticity confirmed by demonstrating high affinity hormone binding and reactivity to monoclonal ***antibody*** 9A7 gamma. The expressed hVDR displays strict dependence on the family of retinoid X receptors (RXRs) for binding to the vitamin D-responsive element (VDRE) in the rat osteocalcin gene. Similar overexpression in E. coli of the DNA binding domain (delta 134), containing only residues 4-133 of hVDR, generated a receptor species that possesses intrinsic DNA binding activity. Both full-length and delta 134 hVDRs retain similar DNA binding specificities when tested with several natural hormone responsive elements, indicating that the N-terminal zinc finger region determines hVDR-DNA sequence selectivity. The C-terminal region of the molecule is required for hormone binding and confers the receptor with the property of very high affinity DNA binding, via ***heterodimerization*** between hVDR and RXR. A natural ligand for the RXR co-receptor, 9-cis retinoic acid, suppresses both VDR-RXR binding to the VDRE and 1,25(OH)2D3 stimulated transcription, indicating that 9-cis retinoic acid recruits RXR away from VDR to instead form RXR homodimers.

L47 ANSWER 100 OF 132 MEDLINE
ACCESSION NUMBER: 96129484 MEDLINE
DOCUMENT NUMBER: 96129484 PubMed ID: 8581386
TITLE: Toward the production of ***bispecific*** ***antibody*** fragments for clinical applications.
AUTHOR: Carter P; Ridgway J; Zhu Z
CORPORATE SOURCE: Department of Molecular Oncology, Genentech Inc., South San Francisco, CA 94080, USA.
SOURCE: JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 463-70. Ref: 63
Journal code: 9306048. ISSN: 1061-6128.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960327
Last Updated on STN: 19960327
Entered Medline: 19960320

AB The clinical potential of ***bispecific*** antibodies (BsAb) has been hindered by the difficulty of obtaining clinical grade material, together with the immunogenicity of rodent-derived BsAb in patients. The supply issue is being directly addressed by ***recombinant*** methods for BsAb fragment production reviewed here. The immunogenicity issue will likely be overcome by the use of humanized or human antibodies. Currently, three technologies appear suitable for the production of BsAb fragments for clinical applications: BsF(***ab*** ')2 ***assembled*** from Fab' fragments expressed in Escherichia coli, BsF(***ab*** ')2 ***assembled*** using leucine zippers, and diabodies.

L47 ANSWER 101 OF 132 MEDLINE
ACCESSION NUMBER: 96129471 MEDLINE
DOCUMENT NUMBER: 96129471 PubMed ID: 8581373
TITLE: Expression of monovalent and bivalent ***antibody*** fragments in Escherichia coli.
AUTHOR: Grant S D; Cupit P M; Learmonth D; Byrne F R; Graham B M; Porter A J; Harris W J
CORPORATE SOURCE: Department of Molecular and Cell Biology, University of Aberdeen, Marischal College, UK.

SOURCE: JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 383-8.
Journal code: 9306048. ISSN: 1061-6128.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960327
Last Updated on STN: 19960327
Entered Medline: 19960320

AB The technology of humanization of rodent antibodies has opened the way for a broad range of therapeutic antibodies with very low immunogenicity, which are, therefore, suitable for repeated dosing. Such intact antibodies have extended serum half-lives and biodistribution profiles very similar to human antibodies. For some applications, however, the ideal therapeutic should have reduced serum half-life and altered biodistribution patterns typical of ***antibody*** fragments, such as Fab or single chain Fv. ***Bispecific*** ***antibody*** fragments offer exciting additional therapeutic possibilities, but their successful manufacture and purification on a large scale require the development of new methods. ***Antibody*** fragments often ***assemble*** in Escherichia coli as monovalent fragments with reduced affinities. We describe the spontaneous ***assembly*** of bivalent ***antibody*** fragments in E. coli and methods of purification that yield either bivalent or monovalent molecules as required.

L47 ANSWER 102 OF 132 MEDLINE
ACCESSION NUMBER: 1998298518 MEDLINE
DOCUMENT NUMBER: 98298518 PubMed ID: 9634779
TITLE: Calmodulin as a versatile tag for ***antibody*** fragments.
AUTHOR: Neri D; de Lalla C; Petrul H; Neri P; Winter G
CORPORATE SOURCE: Cambridge Centre for Protein Engineering, MRC Centre, UK.
SOURCE: BIO/TECHNOLOGY, (1995 Apr) 13 (4) 373-7.
Journal code: 8309273. ISSN: 0733-222X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980731
Last Updated on STN: 19980731
Entered Medline: 19980717

AB Calmodulin is a highly acidic protein (net charge -24 at pH 8.0 in the absence of calcium) that binds to peptide and organic ligands with high affinity (Ka > 10(9) M-1) in a calcium-dependent manner. We have exploited these properties to develop calmodulin as a versatile tag for ***antibody*** fragments. Fusions of calmodulin with single chain Fv fragments (scFv) could be expressed by secretion from bacteria in good yield (5-15 mg/l in shaker flasks), and purified from periplasmic lysates or broth to homogeneity in a single step, either by binding to anion-exchange resin (DEAE-Sephadex), or to an organic ligand of calmodulin (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide-agarose). The ***antibody*** fusions could be detected by binding of fluorescently labeled peptide ligands, as illustrated by their use in confocal microscopy, fluorescent activated cell sorting and "band shift" gel electrophoresis. Moreover, the interaction between calmodulin and peptide ligands could provide a means of ***heterodimerization*** of proteins, as illustrated by the ***assembly*** of an ***antibody*** -calmodulin fusion with maltose binding protein tagged with a peptide ligand of calmodulin.

L47 ANSWER 103 OF 132 MEDLINE
ACCESSION NUMBER: 95182447 MEDLINE
DOCUMENT NUMBER: 95182447 PubMed ID: 7533215
TITLE: High-affinity antigen binding by chelating ***recombinant*** antibodies (CRAbs).

AUTHOR: Neri D; Momo M; Prospero T; Winter G
CORPORATE SOURCE: Cambridge Centre for Protein Engineering, MRC Centre, England.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1995 Feb 24) 246 (3) 367-73. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950419
Last Updated on STN: 19960129
Entered Medline: 19950404
AB We have developed a strategy for making ***antibody*** fragments with high binding affinities by harnessing the chelate effect. We create a ***bispecific*** ***antibody*** fragment (Chelating ***Recombinant*** ***Antibody*** or CRAb) that recognizes adjacent and non-overlapping epitopes of the target antigen, and is flexible enough to bind to both epitopes simultaneously. Here the strategy is illustrated with two antibodies that form complexes of known three-dimensional structure against different epitopes of lysozyme. Computer graphic modelling indicated that two single-chain ***antibody*** fragments (scFv) derived from antibodies D1.3 (Ka = 10(8) M-1) and mutant HyHEL-10 (Ka = 10(6) M-1) could be linked together on the surface of lysozyme by a flexible and hydrophilic polypeptide between the C terminus of one fragment and the N terminus of the other. The CRAb gene was ***assembled*** and the CRAb expressed by secretion from bacteria. The purified CRAb was shown to have a much higher affinity than either of the scFv fragments, as shown by competition ELISA (Kd > 10(9) M-1), bandshift on gels (Ka > 2 x 10(9) M-1) and fluorescence quench (Ka > 1.3 x 10(10) M-1).

L47 ANSWER 104 OF 132 MEDLINE
ACCESSION NUMBER: 95121810 MEDLINE
DOCUMENT NUMBER: 95121810 PubMed ID: 7821762
TITLE: Single-chain Fvs.
AUTHOR: Raag R; Whitlow M
CORPORATE SOURCE: Department of Chemistry, University of California at Berkeley 94720.
SOURCE: FASEB JOURNAL, (1995 Jan) 9 (1) 73-80. Ref: 47
Journal code: 8804484. ISSN: 0892-6638.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950223
Last Updated on STN: 19950223
Entered Medline: 19950216
AB Single-chain Fvs (sFvs) are ***recombinant*** ***antibody*** fragments consisting of only the variable ***light*** chain (VL) and variable ***heavy*** chain (VH) domains covalently connected to one another by a polypeptide linker. Due to their small size, sFvs have rapid pharmacokinetics and tumor penetration in vivo. Single-chain Fvs also show a concentration-dependent tendency to oligomerize. Bivalent sFvs are formed when the variable domains of a sFv disassociate from one another and reassociate with the variable domains of a second sFv. Similar rearrangement and reassociation of variable domains from different sFvs can result in the formation of trimers or higher multimeric oligomers. Each Fv in a bivalent or multivalent Fv is composed of the VL domain from one sFv and the VH domain from a second sFv. Modifying linker length or the inclusion of antigen may stabilize the VL/VH ***interface*** against rearrangement such that specific multimeric or monomeric forms of sFvs may be isolated. Nuclear magnetic resonance studies have shown that McPC603-derived Fv and sFvs have similar structures, and that the sFv

at 0.6 M NaCl solution.

L47 ANSWER 108 OF 132 MEDLINE
ACCESSION NUMBER: 94246169 MEDLINE
DOCUMENT NUMBER: 94246169 PubMed ID: 7514636
TITLE: Functionally important amino acids in the TCR revealed by immunoselection of membrane TCR-negative T cells.
AUTHOR: Caspar-Bauguil S; Arnaud J; Gouaillard C; Hou X; Geisler C; Rubin B
CORPORATE SOURCE: Immunopathology and Human Genetics Center (CIGH-CNRS), Toulouse, France.
SOURCE: JOURNAL OF IMMUNOLOGY, (1994 Jun 1) 152 (11) 5288-98. Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940629
Last Updated on STN: 19960129
Entered Medline: 19940621
AB A spontaneous TCR cell surface variant (3P11) of the Jurkat T cell line is described and characterized. 3P11 was selected by incubation of Jurkat cells with anti-TCR ***mAb*** followed by passage through Ig anti-Ig columns and cloning. 3P11 contained mRNA for both Ti alpha and Ti beta and CD3 gamma, delta, epsilon and zeta. Biochemical analyses demonstrated that all of the TCR components were produced in 3P11 cells. The Ti alpha beta/CD3 gamma delta epsilon zeta complex was ***assembled*** in the endoplasmic reticulum but the zeta did not associate with this complex. Epitopes recognized by the Ti beta ***chain*** specific ***mAb*** beta F1 and JOVI as well as anti-V beta 8 were affected in the 3P11 Ti beta ***chain*** indicating that the 3P11 Ti beta ***chain*** was mutated. Transfection of a wild-type Ti beta cDNA into 3P11 cells reconstituted TCR expression. Sequence analyses of the 3P11 Ti beta ***chain*** demonstrated a guanine to adenine change in the second nucleotide of the triplet coding for cysteine191 resulting in a cysteine to ***tyrosine*** exchange. Cysteine191 is the C-terminal cysteine involved in the intrachain disulfide bond in the C ***domain*** of the Ti beta ***chain*** ; thus, the 3P11 Ti beta ***chain*** did not contain this disulfide bond. Transfection of a site-directed Ti beta ***chain*** containing the 3P11 mutation into a Ti beta negative variant of the Jurkat cell line resulted in a TCR phenotype identical with 3P11 demonstrating that the mutation identified in the 3P11 Ti beta ***chain*** was the sole cause for the 3P11 defect.

L47 ANSWER 109 OF 132 MEDLINE
ACCESSION NUMBER: 95107943 MEDLINE
DOCUMENT NUMBER: 95107943 PubMed ID: 7809029
TITLE: ***Multimerization*** behaviour of single chain Fv variants for the tumour-binding ***antibody*** B72.3.
AUTHOR: Desplancq D; King D J; Lawson A D; Mountain A
CORPORATE SOURCE: Oncology Department, Celltech Research, Slough, UK.
SOURCE: PROTEIN ENGINEERING, (1994 Aug) 7 (8) 1027-33. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19950215
Entered Medline: 19950127
AB A systematic study has been performed on the relationship between linker length, relative orientation of variable domains, ***multimerization*** behaviour and antigen binding activity for single chain Fvs (scFvs) of the tumour-binding ***antibody*** B72.3. Thirteen scFv variants with linkers comprising up to six repeats of the motif Gly-Gly-Gly-Gly-Ser were

studied. All these scFvs showed a tendency to form dimers or higher molecular weight species, and this tendency decreased with increasing linker length. The dimers and higher molecular weight forms may arise from head to tail intermolecular association of VH and VL domains. For each linker length, scFvs with the organization VL-linker-VH showed greater binding activity than those with the organization VH-linker-VL. In fact, for the latter organization only the variant with a 30 amino acid linker showed good binding activity, suggesting that (i) for B72.3 the C-terminus of VH or the N-terminus of VL makes a structural contribution to antigen binding, and (ii) shorter linkers interfere with this contribution. Antigen binding studies on scFvs should be interpreted with caution because of their tendency to ***multimerize*** . Such ***multimerization*** can be minimized by using linkers longer than those in common use.

L47 ANSWER 110 OF 132 MEDLINE
ACCESSION NUMBER: 95107942 MEDLINE
DOCUMENT NUMBER: 95107942 PubMed ID: 7809028
TITLE: Multivalent Fvs: characterization of single-chain Fv oligomers and preparation of a ***bispecific*** Fv.
AUTHOR: Whitlow M; Filpula D; Rollence M L; Feng S L; Wood J F
CORPORATE SOURCE: Research and Development Department, Enzon, Incorporated, Piscataway, NJ 08854-3998.
SOURCE: PROTEIN ENGINEERING, (1994 Aug) 7 (8) 1017-26. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19980206
Entered Medline: 19950127

AB Single-chain Fv proteins are known to aggregate and form multimeric species. We report here that these molecules represent a new class of molecular ***assembly*** , which we have termed multivalent Fvs. Each binding site in a multivalent Fv comprises the variable light-chain (VL) domain from a single-chain Fv, and the variable heavy-chain (VH) domain from a second single-chain Fv. Each single-chain Fv in a multivalent Fv is part of two binding sites. We have characterized the multivalent forms of the 4-4-20, CC49 and B6.2 sFvs. The degree of multivalent Fv formation is linker-dependent. Multivalent Fvs cannot form in the absence of an intact linker. Multivalent Fvs can be stabilized by their antigen. The conversion between different forms of the multivalent Fvs can be catalyzed by disassociating agents such as 0.5 M guanidine hydrochloride with 20% ethanol. Multivalent Fvs have significantly different stabilities depending on the specific variable domains from which they are constructed. Two models have been proposed for the structure of a multivalent Fv. We have tested each model by attempting to produce a heterodimer from the anti-fluorescein 4-4-20 and anti-tumor CC49 variable regions. We successfully produced a 4-4-20/CC49 heterodimer that comprises two mixed sFvs. The first mixed sFv is composed of the 4-4-20 VL domain, a 12 residue linker and the CC49 Vh domain. The second mixed sFv is composed of a CC49 VL domain, a 12 residue linker and the 4-4-20 VH domain. The 4-4-20/CC49 heterodimer bound both fluorescein and the tumor-associated glycoprotein-72 antigen. These results support a VH/VL 'rearrangement' model in which each variable domain of a multivalent Fv binding site comes from a different polypeptide chain.

L47 ANSWER 111 OF 132 MEDLINE
ACCESSION NUMBER: 94156049 MEDLINE
DOCUMENT NUMBER: 94156049 PubMed ID: 8112468
TITLE: 'Camelising' human ***antibody*** fragments: NMR studies on VH domains.
AUTHOR: Davies J; Riechmann L
CORPORATE SOURCE: MRC Laboratory of Molecular Biology, Cambridge, UK.
SOURCE: FEBS LETTERS, (1994 Feb 21) 339 (3) 285-90.

supporting previous evidence that pro alpha 3(XI) and pro alpha 1(II) have the same primary structure. When cultured in the presence of 50 mM ***arginine*** , IRC cells could be induced to synthesize pro alpha 1(II) chains in excess over pro alpha 1(XI) and pro alpha 2(XI). Only under these conditions were type II collagen molecules detected, suggesting a preferential association of pro alpha 1(II) with the pro alpha 1 and/or pro alpha 2 chains of type XI collagen.

L47 ANSWER 113 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94356411 EMBASE
DOCUMENT NUMBER: 1994356411
TITLE: Mechanism of B cell antigen receptor function: Transmembrane signaling and triggering of apoptosis.
AUTHOR: DeFranco A.L.; Mittelstadt P.R.; Blum J.H.; Stevens T.L.; Law D.A.; Chan - V.W.F.; Foy S.P.; Datta S.K.; Matsuuchi L.
CORPORATE SOURCE: Dept. of Microbiology/Immunology, G. W. Hooper Foundation, University of California,San Francisco, CA, United States
SOURCE: Advances in Experimental Medicine and Biology, (1994) 365/- (9-22).
ISSN: 0065-2598 CODEN: AEMBAP
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The antigen receptor of B lymphocytes (BCR) plays important roles in virtually every stage in the development, inactivation, or activation of B cells. The BCR is a complex of membrane immunoglobulin (mIg) and a heterodimer of two transmembrane polypeptides called Ig-.alpha. and Ig-.beta.. Site directed mutation of the .mu. immunoglobulin ***heavy*** ***chain*** has demonstrated that the .mu. transmembrane ***domain*** plays a key role in the ***assembly*** of mIgM with Ig-.alpha./Ig-.beta.. In addition, there is a strong correlation between the ability of various mutant mIgM molecules to associate with Ig-.alpha./Ig-.beta. and their ability to induce signal transduction reactions such as protein ***tyrosine*** phosphorylation and phosphoinositide breakdown. The cytoplasmic domains of Ig-.alpha. and Ig-.beta. share a region of limited homology with each other and with components of the T cell antigen receptor and of the Fc receptor. The presence of regions of the cytoplasmic domains of Ig-.alpha. or Ig-.beta. including this conserved amino acid sequence motif is sufficient to confer signaling function on chimeric transmembrane proteins. Both Ig-.alpha. and Ig-.beta. chimeras are capable of inducing all of the BCR signaling events tested. Based on these and related observations, we propose that the motifs act to initiate the BCR signaling reactions by binding and activating ***tyrosine*** kinases. Among the important events mediated by BCR signaling is induced expression of a series of genes referred to as early response genes. In B cells these include transcription factors and at least one component that regulates signaling events. One of these genes, c-myc, appears to play an important role in mediating apoptosis in B cells stimulated via the BCR complex.

L47 ANSWER 114 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:537321 CAPLUS
DOCUMENT NUMBER: 119:137321
TITLE: ***Bispecific*** antibody heterodimer production with leucine zipper
INVENTOR(S): Tso, J. Yun; Kostelny, Sheri A.; Cole, Michael S.
PATENT ASSIGNEE(S): Protein Design Labs, Inc., USA
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9311162	A1	19930610	WO 1992-US10140	19921125
W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
US 5932448	A	19990803	US 1991-801798	19911129
AU 9331472	A1	19930628	AU 1993-31472	19921125
EP 618929	A1	19941012	EP 1992-925401	19921125
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 07501698	T2	19950223	JP 1992-510218	19921125
AU 9674118	A1	19970220	AU 1996-74118	19961202
AU 682141	B2	19970918		

PRIORITY APPLN. INFO.: US 1991-801798 19911129
WO 1992-US10140 19921125

AB Methods are disclosed for producing and using ***bispecific*** antibodies formed by leucine zippers. Leucine zippers capable of preferentially forming heterodimers are resp. linked to epitope-binding components comprising different binding specificities. ***Bispecific*** antibodies are formed by pairwise assocn. of the leucine zippers, forming a heterodimer which links the 2 distinct epitope-binding components. ***Heterodimerization*** can occur by interaction of the 2 leucine zipper regions, forming a ***bispecific*** antibody. Such a ***bispecific*** antibody may be further stabilized by the formation of intermol. bonds, e.g. disulfide bonds, between the 2 monomeric subunits. Subsequent to the formation of such intermol. bonds between the monomeric subunits, the leucine zippers may be removed or retained. ***Bispecific*** antibodies produced by these methods are substantially pure and may be produced in high yields and on a large scale. Alternatively, bifunctional heterodimers may be formed by linking an epitope-binding component to a macromol. species that is not an epitope-binding component. Thus, PCR methodol. was used to join the Fos or Jun leucine zipper sequence to the 1st codon of the CH2 exon of a mouse IgG2a gene. At the fusion junctions, 2 ***glycine*** codons were introduced to make the joints more flexible in the protein products. After the leucine zipper sequences, a stop codon and a sequence contg. the polyadenylation signal from the mouse IgG2a gene were included. The gene fusions were sep. inserted into an expression vector previously used for heavy-chain synthesis. Into the Jun expression plasmid was inserted the VH gene for the mouse anti-Tac antibody (binding to the p55 chain of the human interleukin-2 receptor), and into the Fos plasmid was inserted the VH gene of the hamster 145-2C11 antibody (recognizing the .epsilon. chain of the mouse CD3 complex). Each VH gene included the signal sequences and J segment and was followed by a splice donor sequence to allow splicing to the CH1 domain. Analogous plasmids were prepd. that resp. contained the VL genes of anti-Tac and 145-2C11 together with the mouse V.kappa. gene, and plasmids were transfected into Sp2/0 murine myeloma cells.

L47 ANSWER 115 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93150248 EMBASE
DOCUMENT NUMBER: 1993150248
TITLE: A cationic region of the platelet-derived growth factor (PDGF) A- ***chain*** (Arg159-Lys160-Lys161) is required for receptor binding and mitogenic activity of the PDGF-AA homodimer.
AUTHOR: Fenstermaker R.A.; Poptic E.; Bonfield T.L.; Knauss T.C.; Corsillo L.; Piskurich J.F.; Kaetzel C.S.; Jentoft J.E.; Gelfand C.; DiCorleto P.E.; Kaetzel Jr. D.M.
CORPORATE SOURCE: Research Division, Veterans Affairs Medical Ctr. Dept., 10701 E. Blvd.,Cleveland, OH 44106, United States
SOURCE: Journal of Biological Chemistry, (1993) 268/14 (10482-10489).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English
SUMMARY LANGUAGE: English
AB Platelet-derived growth factor-AA and -BB homodimers and - ***AB*** heterodimers bind with high affinity to the platelet-derived growth factor (PDGF) .alpha.- receptor. Basic polypeptides such as polylysine and protamine sulfate compete with PDGF for receptor binding, suggesting a role for ligand positive charge in the binding interaction. A pentapeptide amino acid sequence with a cationic tripeptide core is perfectly conserved between the A- and B-chains (Val158-Arg159-Lys160-Lys161-Pro162) and was therefore considered as a possible .alpha.-receptor-binding ***domain*** . We have investigated the functional importance of positive charge within this region of the PDGF A- ***chain*** by using site-directed mutagenesis to convert the cationic core amino acids to the acidic sequence triglutamic acid. cDNAs encoding wild-type (PDGF-AA(wt)) and charge mutant (PDGF-AA(cm)) proteins were expressed following stable transfection of Chinese hamster ovary cells. Proper ***assembly*** and secretion of PDGF-AA(cm) was verified by metabolic labeling with [35S]cysteine, immunoprecipitation, and SDS-polyacrylamide gel electrophoresis analysis under nonreducing and reducing conditions. PDGF-AA(cm) was secreted as two major species of disulfide-linked A- ***chain*** homodimers identical in molecular mass to those observed for PDGF-AA(wt) (32 and 35 kDa). Secreted PDGF-AA(wt) and PDGF-AA(cm) proteins were purified to homogeneity and subjected to structural and functional analyses. Compared to purified PDGF-AA(wt), PDGF-AA(cm) displayed a marked reduction in both binding affinity for PDGF .alpha.-receptors and mitogenic activity in Swiss 3T3 cells. Large reductions were also observed in the ability of semi-purified PDGF-AA(cm) to stimulate calcium influx and the production of inositol phosphates. Measurement of circular dichroism spectra of highly purified PDGF-AA(cm) and PDGF-AA(wt) revealed no significant difference in secondary structure. Collectively, these results indicate that the cationic Arg159- Lys160-Lys161 region plays a critical role in the biological activity of PDGF-AA by direct participation in ligand binding to the PDGF .alpha.-receptor.

L47 ANSWER 116 OF 132 MEDLINE
ACCESSION NUMBER: 93361022 MEDLINE
DOCUMENT NUMBER: 93361022 PubMed ID: 8395017
TITLE: Retinoid X receptors stimulate and 9-cis retinoic acid inhibits 1,25-dihydroxyvitamin D3-activated expression of the rat osteocalcin gene.
AUTHOR: MacDonald P N; Dowd D R; Nakajima S; Galligan M A; Reeder M C; Haussler C A; Ozato K; Haussler M R
CORPORATE SOURCE: Department of Biochemistry, University of Arizona College of Medicine, Tuscon 85724.
CONTRACT NUMBER: 1-F32-GM-13846 (NIGMS)
AR-15781 (NIAMS)
DK-33351 (NIDDK)
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1993 Sep) 13 (9) 5907-17.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19931008
Last Updated on STN: 19970203
Entered Medline: 19930923
AB The vitamin D receptor (VDR) binds the vitamin D-responsive element (VDRE) as a heterodimer with an unidentified receptor auxiliary factor (RAF) present in mammalian cell nuclear extracts. VDR also interacts with the retinoid X receptors (RXRs), implying that RAF may be related to the RXRs. Here we demonstrate that highly purified HeLa cell RAF contained RXR beta immunoreactivity and that both activities copurified and precisely coeluted in high-resolution hydroxylapatite chromatography. Furthermore, an RXR beta-specific ***antibody*** disrupted VDR-RAF-VDRE complexes in mobility shift assays. These data strongly indicate that HeLa RAF is highly related to or is identical to RXR beta. Consequently, the effect of

the 9-cis retinoic acid ligand for RXRs was examined in 1,25-dihydroxyvitamin D3 [1,25(OH)2D3]-activated gene expression systems. Increasing concentrations of 9-cis retinoic acid (1 nM to 1 microM) markedly reduced 1,25(OH)2D3-dependent accumulation of osteocalcin mRNA in osteoblast-like ROS 17/2.8 cells. All-trans retinoic acid also interfered with vitamin D responsiveness, but it was consistently less potent than the 9-cis isomer. Transient transfection studies revealed that attenuation by 9-cis retinoic acid was at the transcriptional level and was mediated through interactions at the osteocalcin VDRE. Furthermore, overexpression of both RXR beta and RXR alpha augmented 1,25(OH)2D3 responsiveness in transient expression studies. Direct analysis of VDRE binding in mobility shift assays demonstrated that heteromeric interactions between VDR and RXR were enhanced by 1,25(OH)2D3 and were not affected appreciably by 9-cis retinoic acid, except that inhibition was observed at high retinoid concentrations. These data suggest a regulatory mechanism for osteocalcin gene expression that involves 1,25(OH)2D3-induced ***heterodimerization*** of VDR and unliganded RXR. 9-cis retinoic acid may attenuate 1,25(OH)2D3 responsiveness by diverting RXRs away from VDR-mediated transcription and towards other RXR-dependent transcriptional pathways.

L47 ANSWER 117 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93187633 EMBASE
DOCUMENT NUMBER: 1993187633
TITLE: Control of fibroblast growth factor receptor kinase signal transduction by ***heterodimerization*** of combinatorial splice variants.
AUTHOR: Shi E.; Kan M.; Xu J.; Wang F.; Hou J.; McKeehan W.L.
CORPORATE SOURCE: W. Alton Jones Cell Sci. Ctr., Inc., 10 Old Barn Road,Lake Placid, NY 12946, United States
SOURCE: Molecular and Cellular Biology, (1993) 13/7 (3907-3918). ISSN: 0270-7306 CODEN: MCEBD4
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A differentiated liver cell (HepG2), which exhibits a dose-dependent growth-stimulatory and growth-inhibitory response to heparin-binding fibroblast growth factor type 1 (FGF-1), displays high- and low-affinity receptor phenotypes and expresses specific combinatorial splice variants .alpha.1, .beta.1, and .alpha.2 of the FGF receptor (FGF-R) gene (flg). The extracellular domains of the .alpha. and .beta. variants consist of three and two immunoglobulin loops, respectively, while the intracellular variants consist of a tyrosine kinase (type 1) isoform and a kinase-defective (type 2) isoform. The type 2 isoform is also devoid of the two major intracellular tyrosine autophosphorylation sites (Tyr-653 and Tyr-766) in the type 1 kinase. An analysis of ligand affinity, dimerization, autophosphorylation, and interaction with src homology region 2 (SH2) substrates of the ***recombinant*** .alpha.1, .beta.1, and .alpha.2 isoforms was carried out to determine whether dimerization of the combinatorial splice variants might explain the dose-dependent opposite mitogenic effects of FGF. Scatchard analysis indicated that the .alpha. and .beta. isoforms exhibit low and high affinity for ligand, respectively. The three combinatorial splice variants dimerized in all combinations. FGF enhanced dimerization and kinase activity, as assessed by receptor autophosphorylation. Phosphopeptide analysis revealed that phosphorylation of Tyr-653 was reduced relative to phosphorylation of Tyr-766 in the type 1 kinase component of heterodimers of the type 1 and type 2 isoforms. The SH2 domain substrate, phospholipase C.gamma.1 (PLC.gamma.1), associated with the phosphorylated type 1-type 2 heterodimers but was phosphorylated only in preparations containing the type 1 kinase homodimer. The results suggest that phosphorylation of Tyr-653 within the kinase catalytic domain, but not Tyr- 766 in the COOH-terminal domain, may be stringently dependent on a trans intermolecular mechanism within FGF-R kinase homodimers. Although

phosphotyrosine 766 is sufficient for interaction of PLC.gamma.1 and other SH2 substrates with the FGF-R kinase, phosphorylation and presumably activation of substrates require the kinase homodimer and phosphorylation of Tyr-653. We propose that complexes of phosphotyrosine 766 kinase monomers and SH2 domain signal transducers may constitute unactivated presignal complexes whose active or inactive fate depends on homodimerization with a kinase or ***heterodimerization*** with a kinase-defective monomer, respectively. The results suggest a mechanism for control of signal transduction by different concentrations of ligand through ***heterodimerization*** of combinatorial splice variants from the same receptor gene.

L47 ANSWER 118 OF 132 MEDLINE
ACCESSION NUMBER: 93285105 MEDLINE
DOCUMENT NUMBER: 93285105 PubMed ID: 8389696
TITLE: RARs and RXRs: evidence for two autonomous transactivation functions (AF-1 and AF-2) and ***heterodimerization*** in vivo.
AUTHOR: Nagpal S; Friant S; Nakshatri H; Chambon P
CORPORATE SOURCE: Laboratoire de Genetique Moleculaire des Eucaryotes du CNRS, Strasbourg, France.
SOURCE: EMBO JOURNAL, (1993 Jun) 12 (6) 2349-60.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930715
AB We have previously reported that the ***AB*** regions of retinoic acid receptors (RARs and RXRs) contain a transcriptional activation function capable of modulating the activity of the ligand-dependent activation function present in the C-terminal DE regions of these receptors. However, we could not demonstrate that these ***AB*** regions possess an autonomous activation function similar to the AF-1s found in the ***AB*** regions of steroid hormone receptors. Using the mouse CRBPII promoter as a reporter gene, we now report that the ***AB*** regions of RAR alpha, beta and gamma, as well as those of RXR alpha and gamma, contain an autonomous, ligand-independent activation function, AF-1, which can efficiently synergize with AF-2s. Moreover, AF-1s account for the ligand-independent, constitutive activation of transcription by RXR alpha and gamma. We also show that RARs and RXRs preferentially ***heterodimerize*** in solution in cultured cells in vivo, through the dimerization interface present in their E region, irrespective of the presence of all-trans or 9-cis retinoic acid. Furthermore, our results indicate that homodimeric interactions are not observed in cultured cells in vivo under conditions where heterodimeric interactions readily occur, which is in agreement with previous observations showing the preferential binding of RAR-RXR heterodimers to RA response elements in vitro.

L47 ANSWER 119 OF 132 MEDLINE
ACCESSION NUMBER: 93180783 MEDLINE
DOCUMENT NUMBER: 93180783 PubMed ID: 8441384
TITLE: cDNA cloning of transcription factor E4TF1 subunits with Ets and notch motifs.
AUTHOR: Watanabe H; Sawada J; Yano K; Yamaguchi K; Goto M; Handa H
CORPORATE SOURCE: Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan.
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1993 Mar) 13 (3) 1385-91.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D13316; GENBANK-D13317; GENBANK-D13318

DOCUMENT NUMBER: 118:35073
TITLE: Sequences directing dihydrolipoamide dehydrogenase (E3) binding are located on the 2-oxoglutarate dehydrogenase (E1) component of the mammalian 2-oxoglutarate dehydrogenase multienzyme complex
AUTHOR(S): Rice, Jacqueline E.; Dunbar, Bryan; Lindsay, J. Gordon
CORPORATE SOURCE: Dep. Biochem., Univ. Glasgow, Glasgow, G12 8QQ, UK
SOURCE: EMBO Journal (1992), 11(9), 3229-35
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sequences located in the N-terminal region of the high Mr 2-oxoglutarate dehydrogenase (E1) enzyme of the mammalian 2-oxoglutarate dehydrogenase multienzyme complex (OGDC) exhibit significant similarity with corresponding sequences from the lipoyl domains of the dihydrolipoamide acetyltransferase (E2) and protein X components of eukaryotic pyruvate dehydrogenase complexes (PDCs). Two addnl. features of this region of E1 resemble lipoyl domains: (i) it is readily released by trypsin, generating a small N-terminal peptide with an apparent Mr of 10,000 and a large stable 100,000 Mr fragment (E1') and (ii) it is highly immunogenic, inducing the bulk of the ***antibody*** response to intact E1. This lipoyl-like ***domain*** lacks a functional lipoamide group. Selective but extensive degrdn. of E1 with proteinase ***Arg*** C or specific conversion of E1 to E1' with trypsin both cause loss of overall OGDC function although the E1' fragment retains full catalytic activity. Removal of this small N-terminal peptide promotes the dissocn. of dihydrolipoamide dehydrogenase (E3) from the E2 core ***assembly*** and also affects the stability of E1 interaction. Thus, structural roles which are mediated by a specific gene product, protein X in PDC and possibly also the E2 subunit, are performed by similar structural elements located on the E1 enzyme of the OGDC.

L47 ANSWER 123 OF 132 MEDLINE
ACCESSION NUMBER: 92144568 MEDLINE
DOCUMENT NUMBER: 92144568 PubMed ID: 1737014
TITLE: Miniantibodies: use of amphipathic helices to produce functional, flexibly linked dimeric FV fragments with high avidity in Escherichia coli.
AUTHOR: Pack P; Pluckthun A
CORPORATE SOURCE: Genzentrum Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, FRG.
SOURCE: BIOCHEMISTRY, (1992 Feb 18) 31 (6) 1579-84.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 19920405
Last Updated on STN: 19920405
Entered Medline: 19920317
AB We have designed dimeric ***antibody*** fragments that ***assemble*** in Escherichia coli. They are based on single-chain FV fragments, with a flexible hinge region from mouse IgG3 and an amphiphilic helix fused to the C-terminus of the ***antibody*** fragment. The sequence of the helix was taken either from that of a previously reported four-helix bundle design or from a leucine zipper, optionally extended with a short cysteine-containing peptide. The bivalent fragments associate in vivo, either with covalent linkage or with a monomer-dimer equilibrium, and results from ultracentrifugation sedimentation studies and SDS-PAGE are consistent with dimers. All constructs are able to bind to surface-bound antigen under conditions in which only bivalent but not monovalent ***antibody*** fragments bind. The covalent bundle helix construct shows binding characteristics nearly identical to those of the much larger whole mouse ***antibody*** , resulting in substantially more stable immunoglobulin-antigen complexes than in the case of monovalent fragments. This modular design of natural and engineered

L47 ANSWER 1 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:539851 CAPLUS
DOCUMENT NUMBER: 137:108306
TITLE: ***Assembly*** and screening of highly complex repertoires of antibodies or other proteins showing somatic variation in yeast
INVENTOR(S): Zhu, Li; Hua, Shaobing Benjamin; Sheridan, James; Lin, Yu-Huei
PATENT ASSIGNEE(S): Genetastix Corporation, USA
SOURCE: PCT Int. Appl., 202 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002055718	A2	20020718	WO 2001-US51044	20011031

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-703399 A1 20001031
AB Compns., methods, and kits are provided for efficiently generating and screening a library of highly diverse protein complexes for their ability to bind to other proteins or oligonucleotide sequences. In one aspect of the invention, a library of expression vectors is provided for expressing the library of protein complexes. The library comprises a first nucleotide sequence encoding a first polypeptide subunit; and a second nucleotide sequence encoding a second polypeptide subunit. The first and second nucleotide sequences each independently varies within the library of expression vectors. In addn., the first and second polypeptide subunit are expressed as sep. proteins which self- ***assemble*** to form a protein complex, such as a double- ***chain*** ***antibody*** fragment (dcFv or Fab) and a fully ***assembled*** ***antibody*** , in cells into which the library of expression vectors are introduced. The library of expression vectors can be efficiently generated in yeast cells through homologous recombination; and the encoded proteins complexes with high binding affinity to their target mol. can be selected by high throughput screening in vivo or in vitro.

L47 ANSWER 2 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:256503 CAPLUS
DOCUMENT NUMBER: 136:291007
TITLE: Use of phosphorylation site-specific antibodies in method for quantifying protein kinase activity
INVENTOR(S): Reagan, Kevin J.; Schaeffer, Erik; Wang, Jimin
PATENT ASSIGNEE(S): Biosource International, USA
SOURCE: PCT Int. Appl., 40 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002027017	A2	20020404	WO 2001-US30186	20010927

W: CA, JP
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR

PRIORITY APPLN. INFO.: US 2000-235620P P 20000927

AB The invention involves a method for measuring phosphorylation of proteins and, as such, is an indicator of protein kinase activity. The method involves the in vitro phosphorylation of a target protein but subjecting that protein (non-phosphorylated) to reaction mixt. contg. all reagents, including phosphokinase which allow the creation of a phosphorylated form of protein. The phosphorylated protein is measured by contacting it with an ***antibody*** specific for the phosphorylation sites(s). The invention includes antibodies useful in practicing the methods of the invention. The invention particularly relates to phosphorylation of Tau, Rb and EGFR proteins and antibodies specific for the site of phosphorylation of the Tau, Rb or EGFR proteins.

L47 ANSWER 3 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:31655 CAPLUS
DOCUMENT NUMBER: 136:97299
TITLE: Methods of producing ***multispecific*** heterodimeric fusion protein diabodies and uses in diagnosis and therapy
INVENTOR(S): Mertens, Nico; Grooten, Johan
PATENT ASSIGNEE(S): Vlaams Interuniversitair Instituut Voor Biotechnologie Vzw, Belg.
SOURCE: PCT Int. Appl., 33 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002002781	A1	20020110	WO 2001-EP7557	20010629
AU 2001070609	A5	20020114	AU 2001-70609	20010629

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: EP 2000-202306 A 20000630
WO 2001-EP7557 W 20010629

AB The invention discloses the prodn. of bispecific or ***multispecific*** , bi- or tetravalent antibodies using ***recombinant*** DNA methods and ***recombinant*** prodn. methods. The resulting ***antibody*** consists of one or two diabody mols. that are ***heterodimerized*** by creating a fusion protein with the CL and CH1 Ig const. domains. In particular, each of the chains of the novel heterodimer contains a fusion protein that consists of one or more diabody chains that are coupled to the CL or the CH1 const. Ig domain and forms the formula VH(A)-VL(B)-CL:VH(B)-VL(A)-CH1, where the diabody chains can either be fused at the C-terminus or N-terminus of the Ig const. domain and the order of VH-VL can be reversed. The invention further discloses methods for making these novel heterodimers, DNA comprising genes encoding these novel fusion proteins, transformed host cells, and uses of the fusion proteins for diagnosis and therapy.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L47 ANSWER 4 OF 132 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:254928 BIOSIS
DOCUMENT NUMBER: PREV200200254928
TITLE: Complex formation between Tap and p15 affects binding to FG-repeat nucleoporins and nucleocytoplasmic shuttling.
AUTHOR(S): Katahira, Jun; Straesser, Katja; Saiwaki, Takuya; Yoneda,

Yoshihiro (1); Hurt, Ed
CORPORATE SOURCE: (1) Division of Immunology, Section of Cellular Interactions and Morphogenesis, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamadaoka, Suita, Osaka, 565-0871: yyoneda@anat3.med.osaka-u.ac.jp, cg5@ix.urz.uni-heidelberg.de Japan
SOURCE: Journal of Biological Chemistry, (March 15, 2002) Vol. 277, No. 11, pp. 9242-9246. http://www.jbc.org/. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Mammalian Tap-p15 and yeast Mex67p-Mtr2p are conserved and essential mRNA export factor complexes that transport mRNPs through the nuclear pore. Here, we report that the small subunit p15 affects the binding of the large subunit Tap to repeat nucleoporins. BIAcore measurements revealed that ***recombinant*** Tap binds with high affinity (Kd in the nM range) to repeat nucleoporins and dissociates from them very slowly. In contrast, when ***recombinant*** Tap was bound to p15, the derived heterodimeric complex exhibited a significant lower affinity to FG-repeat nucleoporins (Kd in the muM range). Furthermore, when ***recombinant*** Tap lacking the N-terminal nuclear localization sequences (TapDELTANLS) was microinjected in mammalian cells, it did not shuttle; however, TapDELTANLS with bound p15 efficiently shuttles between nucleus and cytoplasm. We conclude that ***heterodimerization*** of Tap and p15 is required for shuttling of the functional Tap-p15 mRNA exporter complex.

L47 ANSWER 5 OF 132 MEDLINE
ACCESSION NUMBER: 2002219055 MEDLINE
DOCUMENT NUMBER: 21952324 PubMed ID: 11955057
TITLE: Characterization of the protrimer intermediate in the folding pathway of the interdigitated beta-helix tailspike protein.
COMMENT: Erratum in: Biochemistry 2002 Aug 20;41(33):10570
AUTHOR: Benton Christopher B; King Jonathan; Clark Patricia L
CORPORATE SOURCE: Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.
CONTRACT NUMBER: GM17980 (NIGMS)
GM19715 (NIGMS)
SOURCE: BIOCHEMISTRY, (2002 Apr 23) 41 (16) 5093-103.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020417
Last Updated on STN: 20020907
Entered Medline: 20020509
AB P22 tailspike is a homotrimeric, thermostable ***adhesin*** that recognizes the O-antigen lipopolysaccharide of Salmonella typhimurium. The 70 kDa subunits include long beta-helix domains. After residue 540, the polypeptide chains change their path and wrap around one another, with extensive interchain contacts. Formation of this interdigitated ***domain*** intimately couples the ***chain*** folding and ***assembly*** mechanisms. The earliest detectable trimeric intermediate in the tailspike folding and ***assembly*** pathway is the protrimer, suspected to be a precursor of the native trimer structure. We have directly analyzed the kinetics of in vitro protrimer formation and disappearance for wild type and mutant tailspike proteins. The results confirm that the protrimer intermediate is an on-pathway intermediate for tailspike folding. Protrimer was originally resolved during tailspike folding because its migration through nondenaturing polyacrylamide gels was significantly retarded with respect to the migration of the native tailspike trimer. By comparing protein mobility versus acrylamide concentration, we find that the retarded mobility of the protrimer is due exclusively to a larger overall size than the native trimer, rather than an altered net surface charge. Experiments with mutant tailspike proteins

indicate that the conformation difference between protrimer and native tailspike trimer is localized toward the C-termini of the tailspike polypeptide chains. These results suggest that the transformation of the protrimer to the native tailspike trimer represents the C-terminal interdigitation of the three polypeptide chains. This late step may confer the detergent-resistance, protease-resistance, and thermostability of the native trimer.

L47 ANSWER 6 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2002099433 EMBASE
TITLE: Phospholipid-binding ***domain*** of factor VIII is involved in endothelial cell-mediated activation of factor X by factor IXa.
AUTHOR: Brinkman H.-J.M.; Mertens K.; Van Mourik J.A.
CORPORATE SOURCE: J.A. Van Mourik, Department of Blood Coagulation, CLB, Plesmanlaan 125, 1066 CX Amsterdam, Netherlands. J_van_Mourik@clb.nl
SOURCE: Arteriosclerosis, Thrombosis, and Vascular Biology, (2002) 22/3 (511-516).
Refs: 34
ISSN: 1079-5642 CODEN: ATVBFA
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
025 Hematology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Apparently quiescent, nonapoptotic endothelial cells mediate the activation of factor X by activated factor IX in the presence of its cofactor, activated factor VIII. In a previous study, we reported that during the activation of factor X, the interaction of the cofactor with the endothelial cell membrane clearly differs from the interaction of the cofactor with artificial lipid membranes. In the present study, we identified the peptide ***domain*** of factor VIII involved in the ***assembly*** of the enzyme-cofactor complex on the endothelial cell surface. With the use of monoclonal antibodies against different peptide sequences on the factor VIII light ***chain*** , it was observed that the lipid-binding region of the C2 ***domain*** on the factor VIII light ***chain*** mediates the ***assembly*** of the factor X-activating complex on the endothelial cell surface. In addition, a synthetic peptide that constitutes region Ala2318-Tyr2332 of the C2 ***domain*** and that is known for its ability to inhibit the binding of factor VIII to artificial lipid membranes also showed inhibition of the cofactor activity of factor VIII on endothelial cells. Thus, the carboxy-terminal part of the factor VIII light ***chain*** not only contains sites involved in lipid binding but also contains sites involved in complex ***assembly*** on the endothelial cell membrane.

L47 ANSWER 7 OF 132 MEDLINE
ACCESSION NUMBER: 2002181592 MEDLINE
DOCUMENT NUMBER: 21911627 PubMed ID: 11913981
TITLE: Both the dimerization and immunochemical properties of E-cadherin EC1 ***domain*** depend on ***Trp*** (156) residue.
AUTHOR: Laur Oscar Y; Klingelhofer Jorg; Troyanovsky Regina B; Troyanovsky Sergey M
CORPORATE SOURCE: Division of Dermatology, Washington University Medical School, St. Louis, Missouri 63110, USA.
CONTRACT NUMBER: 1POAR45254-01 (SAMHSA)
AR44016-06 (NIAMS)
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (2002 Apr 1) 400 (1) 141-7.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020401
Last Updated on STN: 20020507
Entered Medline: 20020506
AB Using ***site*** -directed ***mutagenesis*** , we show in this paper that the adhesive ***interface*** detected in cadherin crystals is unlikely to mediate adhesive interaction between myc- and flag-tagged E-cadherin molecules in human A-431 cells. We also found that a critical residue within this ***interface*** , His(233), is part of the epitope for ***mAb*** SHE78-7. This epitope was accessible to the ***antibody*** in the adhesive E-cadherin dimers, which is consistent with uninvolvement of the ***site*** containing His(233) in cell-cell adhesion. However, both the adhesive dimerization and the integrity of the SHE78-7 epitope depended on the same intramolecular interaction between ***Trp*** (156) and its hydrophobic pocket. Our data suggest that this interaction may have an important regulatory function in controlling the surface topology of the NH(2)-terminal ***domain*** of E-cadherin.

L47 ANSWER 8 OF 132 MEDLINE
ACCESSION NUMBER: 2002311004 MEDLINE
DOCUMENT NUMBER: 22050817 PubMed ID: 12054774
TITLE: Crystal structure of the anti-His tag ***antibody*** 3D5 single-chain fragment complexed to its antigen.
AUTHOR: Kaufmann Markus; Lindner Peter; Honegger Annemarie; Blank Kerstin; Tschopp Markus; Capitani Guido; Pluckthun Andreas; Grutter Markus G
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (2002 Apr 19) 318 (1) 135-47.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020611
Last Updated on STN: 20020713
Entered Medline: 20020712
AB The crystal structure of a mutant form of the single-chain fragment (scFv), derived from the monoclonal anti-His tag ***antibody*** 3D5, in complex with a hexahistidine peptide has been determined at 2.7 A resolution. The peptide binds to a deep pocket formed at the ***interface*** of the variable domains of the ***light*** and the ***heavy*** chain, mainly through hydrophobic interaction to aromatic residues and hydrogen bonds to acidic residues. The ***antibody*** recognizes the C-terminal carboxylate group of the peptide as well as the main chain of the last four residues and the last three imidazole side-chains. The crystals have a solvent content of 77% (v/v) and form 70 A-wide channels that would allow the diffusion of peptides or even small proteins. The anti-His scFv crystals could thus act as a framework for the crystallization of His-tagged target proteins. Designed mutations in framework regions of the scFv lead to high-level expression of soluble protein in the periplasm of Escherichia coli. The ***recombinant*** anti-His scFv is a convenient detection tool when fused to alkaline phosphatase. When immobilized on a matrix, the ***antibody*** can be used for affinity purification of ***recombinant*** proteins carrying a very short tag of just three histidine residues, suitable for crystallization. The experimental structure is now the basis for the design of antibodies with even higher stability and affinity.

L47 ANSWER 9 OF 132 MEDLINE
ACCESSION NUMBER: 2002366804 IN-PROCESS
DOCUMENT NUMBER: 22106506 PubMed ID: 12111392
TITLE: Human betacellulin structure modeled from other members of EGF family.
AUTHOR: Lopez-Torrejon Ines; Querol Enrique; Aviles Francesc X;

Seno Masaharu; De Llorens Rafael; Oliva Baldomero
CORPORATE SOURCE: Institut de Biotecnologia i de Biomedicina "Vicent Villar Palasi" and Departament de Bioquimica, Universitat Autonoma de Barcelona, 08193 Bellaterra, Barcelona, Spain E-mail: boliva@imim.es Phone: +34 93 5422933 Fax: +34 93 5422802.
SOURCE: J Mol Model (Online), (2002 Apr) 8 (4) 131-44.
Journal code: 9806569. ISSN: 0948-5023.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20020712
Last Updated on STN: 20020712
AB We have modeled betacellulin (BTC) to gain insight into the structural elements that can explain its properties. The epidermal growth factor (EGF) signal transduction pathway, a significant mediator of several cell functions, is based on four closely related ***tyrosine*** kinase receptors. The ErbB receptors are transmembrane glycoproteins and signal transduction is initiated by ligand binding that induces receptor homo- or ***heterodimerization*** to form a complex containing two molecules of ligand and two molecules of receptor. The EGF family of ligands can be divided into three groups based on their ability to bind and activate distinct ErbB receptor homo- and heterodimers. Each member of the group formed by BTC, heparin binding EGF (HB-EGF) and epiregulin (EP) can interact with both the EGF receptor (EGFR) and heregulin receptors (ErbB-3 and ErbB-4), and are hence called " ***bispecific*** " ligands. BTC and EP also present the distinctive feature that they activate all possible heterodimeric ErbB receptors. BTC has been modeled with the program MODELLER, using human EGF, human transforming growth factor alpha (hTGFalpha), human HB-EGF and human heregulin one alpha (hHRG-1alpha) as templates. The structure of the model as well as that of the templates were optimized and a simulation of 100 ps was run for all. The main structural properties of the model and the templates were compared and in conclusion the hBTC conformation was closely similar to that of hTGFalpha. Electronic supplementary material to this paper can be obtained by using the Springer LINK server located at http://dx.doi.org/10.1007/s00894-002-0072-2.

L47 ANSWER 10 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:868530 CAPLUS
DOCUMENT NUMBER: 136:19113
TITLE: Bispecific immunoglobulin-like antigen binding proteins and method of production
INVENTOR(S): Zhu, Zhenping
PATENT ASSIGNEE(S): Imclone Systems Incorporated, USA
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001090192	A2	20011129	WO 2001-US16924	20010524
US 2002103345	A1	20020801	US 2001-865198	20010524

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-206749P P 20000524
AB The present invention is directed to bispecific antigen-binding protein.

These bispecific antigen-binding proteins are optimized in their avidity for antigen(s) but maintain their ability to function as a natural ***antibody*** , including the ability to activate complement mediated cytotoxicity and ***antibody*** dependent cellular toxicity. Natural IgG Igs are monospecific and bivalent, having two binding domains which are specific for the same epitope. By contrast, an IgG type antigen-binding protein of the invention is bispecific and bivalent, having a binding ***domain*** on each ***light*** ***chain*** for one epitope and a binding ***domain*** on each ***heavy*** ***chain*** specific for a second epitope. The design of the present antigen-binding proteins provides for efficient prodn. such that substantially all of the antigen-binding proteins produced are ***assembled*** in the desired configuration.

L47 ANSWER 11 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:338762 CAPLUS
DOCUMENT NUMBER: 134:362292
TITLE: Methods of determining individual hypersensitivity to a pharmaceutical agent from gene expression profile
INVENTOR(S): Farr, Spencer
PATENT ASSIGNEE(S): Phase-1 Molecular Toxicology, USA
SOURCE: PCT Int. Appl., 222 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001032928	A2	20010510	WO 2000-US30474	20001103
WO 2001032928	A3	20020725		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-165398P P 19991105
US 2000-196571P P 20000411

AB The invention discloses methods, gene databases, gene arrays, protein arrays, and devices that may be used to det. the hypersensitivity of individuals to a given agent, such as drug or other chem., in order to prevent toxic side effects. In one embodiment, methods of identifying hypersensitivity in a subject by obtaining a gene expression profile of multiple genes assocd. with hypersensitivity of the subject suspected to be hypersensitive, and identifying in the gene expression profile of the subject a pattern of gene expression of the genes assocd. with hypersensitivity are disclosed. The gene expression profile of the subject may be compared with the gene expression profile of a normal individual and a hypersensitive individual. The gene expression profile of the subject that is obtained may comprise a profile of levels of mRNA or cDNA. The gene expression profile may be obtained by using an array of nucleic acid probes for the plurality of genes assocd. with hypersensitivity. The expression of the genes predetd. to be assocd. with hypersensitivity is directly related to prevention or repair of toxic damage at the tissue, organ or system level. Gene databases arrays and app. useful for identifying hypersensitivity in a subject are also disclosed.

L47 ANSWER 12 OF 132 MEDLINE
ACCESSION NUMBER: 2001522251 MEDLINE
DOCUMENT NUMBER: 21453321 PubMed ID: 11468284
TITLE: Identification of ligand-binding ***site*** III on the

immunoglobulin-like ***domain*** of the granulocyte colony-stimulating factor receptor.
AUTHOR: Layton J E; Hall N E; Connell F; Venhorst J; Treutlein H R
CORPORATE SOURCE: Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch and the Cooperative Research Centre for Cellular Growth Factors, Parkville, Victoria 3050, Australia.. Judy.Layton@ludwig.edu.au
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Sep 28) 276 (39) 36779-87.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200111
ENTRY DATE: Entered STN: 20010925
Last Updated on STN: 20011105
Entered Medline: 20011101
AB The granulocyte colony-stimulating factor receptor (G-CSF-R) forms a tetrameric complex with G-CSF containing two ligand and two receptor molecules. The N-terminal Ig-like ***domain*** of the G-CSF-R is required for receptor dimerization, but it is not known whether it binds G-CSF or interacts elsewhere in the complex. Alanine scanning ***mutagenesis*** was used to show that residues in the Ig-like ***domain*** of the G-CSF-R (***Phe*** (75), Gln(87), and Gln(91)) interact with G-CSF. This binding ***site*** for G-CSF overlapped with the binding ***site*** of a neutralizing anti-G-CSF-R ***antibody*** . A model of the Ig-like ***domain*** showed that the binding ***site*** is very similar to the viral interleukin-6 binding ***site*** (***site*** III) on the Ig-like ***domain*** of gp130, a related receptor. To further characterize the G-CSF-R complex, exposed and inaccessible regions of monomeric and dimeric ligand-receptor complexes were mapped with monoclonal antibodies. The results showed that the E helix of G-CSF was inaccessible in the dimeric but exposed in the monomeric complex, suggesting that this region binds to the Ig-like ***domain*** of the G-CSF-R. In addition, the N terminus of G-CSF was exposed to ***antibody*** binding in both complexes. These data establish that the dimerization ***interface*** of the complete receptor complex is different from that in the x-ray structure of a partial complex. A model of the tetrameric G-CSF.G-CSF-R complex was prepared, based on the viral interleukin-6.gp130 complex, which explains these and previously published data.

L47 ANSWER 13 OF 132 MEDLINE
ACCESSION NUMBER: 2001522246 MEDLINE
DOCUMENT NUMBER: 21453293 PubMed ID: 11451948
TITLE: Crystal structure of a ***recombinant*** anti-estradiol Fab fragment in complex with 17beta -estradiol.
AUTHOR: Lamminmaki U; Kankare J A
CORPORATE SOURCE: Department of Biotechnology, University of Turku, Tykistokatu 6, 6th floor, 20520 Turku, Finland.. urpo.lamminmaki@utu.fi
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Sep 28) 276 (39) 36687-94.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1JGL; PDB-1JHK
ENTRY MONTH: 200111
ENTRY DATE: Entered STN: 20010925
Last Updated on STN: 20011105
Entered Medline: 20011101
AB The crystal structure of a Fab fragment of an anti-17beta-estradiol ***antibody*** 57-2 was determined in the absence and presence of the steroid ligand, 17beta-estradiol (E2), at 2.5 and 2.15-A resolutions,

respectively. The ***antibody*** binds the steroid in a deep hydrophobic pocket formed at the ***interface*** between the variable domains. No major structural rearrangements take place upon ligand binding; however, a large part of the ***heavy*** chain variable domain near the binding pocket is unusually flexible and is partly stabilized when the steroid is bound. The nonpolar steroid skeleton of E2 is recognized by a number of hydrophobic interactions, whereas the two hydroxyl groups of E2 are hydrogen-bonded to the protein. Especially, the 17-hydroxyl group of E2 is recognized by an intricate hydrogen bonding network in which the 17-hydroxyl itself forms a rare four-center hydrogen bond with three polar amino acids; this hydrogen bonding arrangement accounts for the low cross-reactivity of the ***antibody*** with other estrogens such as estrone. The CDRH3 loop plays a prominent role in ligand binding. All the complementarity-determining regions of the ***light*** chain make direct contacts with the steroid, even CDRL2, which is rarely directly involved in the binding of haptens.

L47 ANSWER 14 OF 132 MEDLINE
ACCESSION NUMBER: 2001340955 MEDLINE
DOCUMENT NUMBER: 21216707 PubMed ID: 11278427
TITLE: Peflin and ALG-2, members of the penta-EF-hand protein family, form a heterodimer that dissociates in a Ca2+-dependent manner.
AUTHOR: Kitaura Y; Matsumoto S; Satoh H; Hitomi K; Maki M
CORPORATE SOURCE: Laboratory of Molecular and Cellular Regulation, Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Apr 27) 276 (17) 14053-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010618
Last Updated on STN: 20010618
Entered Medline: 20010614

AB Peflin, a newly identified 30-kDa Ca(2+)-binding protein, belongs to the penta-EF-hand (PEF) protein family, which includes the calpain small subunit, sorcin, grancalcin, and ALG-2 (apoptosis-linked gene 2). We prepared a monoclonal ***antibody*** against human peflin. The ***antibody*** immunoprecipitated a 22-kDa protein as well as the 30-kDa protein from the lysate of Jurkat cells. Western blotting of the immunoprecipitates revealed that the 22-kDa protein corresponds to ALG-2. This was confirmed by Western blotting of the immunoprecipitates of epitope-tagged peflin or ALG-2 whose cDNA expression constructs were transfected to human embryonic kidney (HEK) 293 cells. Gel filtration of the cytosolic fraction of Jurkat cells revealed co-elution of peflin and ALG-2 in fractions eluting earlier than ***recombinant*** ALG-2, further supporting the notion of ***heterodimerization*** of the two PEF proteins. Surprisingly, peflin dissociated from ALG-2 in the presence of Ca(2+). Peflin and ALG-2 co-localized in the cytoplasm, but ALG-2 was also detected in the nuclei as revealed by immunofluorescent staining and subcellular fractionation. Peflin was recovered in the cytosolic fraction in the absence of Ca(2+) but in the membrane/cytoskeletal fraction in the presence of Ca(2+). These results suggest that peflin has features common to those of other PEF proteins (dimerization and translocation to membranes) and may modulate the function of ALG-2 in Ca(2+) signaling.

L47 ANSWER 15 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:634531 CAPLUS
DOCUMENT NUMBER: 136:258038
TITLE: Analysis of the chromosome sequence of the legume symbiont Sinorhizobium meliloti strain 1021
AUTHOR(S): Capela, Delphine; Barloy-Hubler, Frederique; Gouzy,

Jerome; Bothe, Gordana; Ampe, Frederic; Batut, Jacques; Boistard, Pierre; Becker, Anke; Boutry, Marc; Cadieu, Edouard; Dreano, Stephane; Gloux, Stephanie; Godrie, Therese; Goffeau, Andre; Kahn, Daniel; Kiss, Erno; Lelaure, Valerie; Masuy, David; Pohl, Thomas; Portetelle, Daniel; Puhler, Alfred; Purnelle, Benedicte; Ramsperger, Ulf; Renard, Clotilde; Thebault, Patricia; Vandenbol, Micheline; Weidner, Stefan; Galibert, Francis

CORPORATE SOURCE: Laboratoire de Biologie Moleculaire des Relations Plantes-Microorganismes, Unite Mixte de Recherche (UMR) 215 Centre National de la Recherche Scientifique (CNRS), Institut National de la Recherche Agronomique, Chemin, Tolosan, F-31326, Fr.

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2001), 98(17), 9877-9882
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Sinorhizobium meliloti is an .alpha.-proteobacterium that forms agronomically important N2-fixing root nodules in legumes. We report here the complete sequence of the largest constituent of its genome, a 62.7% GC-rich 3654,135-bp circular chromosome. Annotation allowed assignment of a function to 59% of the 3341 predicted protein-coding ORFs, the rest exhibiting partial, weak, or no similarity with any known sequence. Unexpectedly, the level of reiteration within this replicon is low, with only two genes duplicated with more than 90% nucleotide sequence identity, transposon elements accounting for 2.2% of the sequence, and a few hundred short repeated palindromic motifs (RIME1, RIME2, and C) widespread over the chromosome. Three regions with a significantly lower GC content are most likely of external origin. Detailed annotation revealed that this replicon contains all housekeeping genes except two essential genes that are located on pSymB. Amino acid/peptide transport and degrdn. and sugar metab. appear as two major features of the S. meliloti chromosome. The presence in this replicon of a large no. of nucleotide cyclases with a peculiar structure, as well as of genes homologous to virulence determinants of animal and plant pathogens, opens perspectives in the study of this bacterium both as a free-living soil microorganism and as a plant symbiont.

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L47 ANSWER 16 OF 132 MEDLINE

ACCESSION NUMBER: 2001672793 MEDLINE

DOCUMENT NUMBER: 21554792 PubMed ID: 11698279

TITLE: Type 1 von Willebrand disease mutation Cys1149Arg causes intracellular retention and degradation of heterodimers: a possible general mechanism for dominant mutations of oligomeric proteins.

AUTHOR: Bodo I; Katsumi A; Tuley E A; Eikenboom J C; Dong Z; Sadler J E

CORPORATE SOURCE: Howard Hughes Medical Institute and the Department of Medicine, Washington University School of Medicine, St Louis, MO 63110, USA.

CONTRACT NUMBER: DK50053 (NIDDK)

SOURCE: BLOOD, (2001 Nov 15) 98 (10) 2973-9.
Journal code: 7603509. ISSN: 0006-4971.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 200112

ENTRY DATE: Entered STN: 20011126
Last Updated on STN: 20020123
Entered Medline: 20011221

AB Some families affected by von Willebrand disease type 1 show high

penetrance with exceptionally low von Willebrand factor (VWF) levels. Previously, a mutation associated with this dominant phenotype, Cys1149Arg, was found to decrease the secretion of coexpressed normal VWF, and the mutation was proposed to cause intracellular retention of pro-VWF heterodimers. To demonstrate heterodimer formation, a model was developed in which subunits could be distinguished immunologically and by size. ***Recombinant*** VWF lacking domain A1 (dA1), A3 (dA3), or both (dA13) was secreted efficiently as a full range of multimers. Cotransfection of Cys1149Arg and dA13 resulted in the secretion of multimeric VWF containing about 250 kd (Cys1149Arg) and about 210 kd (dA13). Cell lysates contained pro-VWF forms of Cys1149Arg and dA13. Immunoprecipitation with an antidomain A1 ***antibody*** recovered both subunits in heterodimers, and subunit ratios were consistent with random dimerization. Similar results were obtained for cotransfection of Cys1149Arg and dA1. Normal VWF has a Cys1149-Cys1169 intrachain bond. When cotransfected with normal VWF, Cys1149Arg or the double mutant Cys1149Arg+Cys1169Ser caused a similar decrease in VWF secretion, suggesting that an unpaired Cys1169 does not explain the intracellular retention of Cys1149Arg. VWF Cys1149Arg was not secreted from BHK cells but was degraded intracellularly within about 4 hours, and the proteasome inhibitor lactacystin delayed its clearance more than 16 hours. Thus, dominant von Willebrand disease type 1 may be caused by ***heterodimerization*** of mutant and normal subunits in the endoplasmic reticulum followed by proteasomal degradation in the cytoplasm. A similar dominant negative mechanism could cause quantitative deficiencies of other multisubunit proteins.

L47 ANSWER 17 OF 132 MEDLINE
ACCESSION NUMBER: 2001443859 MEDLINE
DOCUMENT NUMBER: 21382459 PubMed ID: 11490003
TITLE: Mutation of a single conserved residue in VH complementarity-determining region 2 results in a severe Ig secretion defect.
AUTHOR: Wiens G D; Lekkerkerker A; Veltman I; Rittenberg M B
CORPORATE SOURCE: Department of Molecular Microbiology and Immunology, Oregon Health Sciences University, Portland, OR 97201, USA.. wiensg@ohsu.edu
CONTRACT NUMBER: AI-14985 (NIAID)
AI-26827 (NIAID)
SOURCE: JOURNAL OF IMMUNOLOGY, (2001 Aug 15) 167 (4) 2179-86. Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20010813
Last Updated on STN: 20020121
Entered Medline: 20011205

AB During an immune response, somatic mutations are introduced into the VH and VL regions of Ig chains. The consequences of somatic mutation in highly conserved residues are poorly understood. Ile51 is present in 91% of murine VH complementarity-determining region 2 sequences, and we demonstrate that single Ile51--> ***Arg*** or Lys substitutions in the PCG1-1 ***Ab*** are sufficient to severely reduce Ig secretion (1-3% of wild-type (WT) levels). Mutant H chains, expressed in the presence of excess L ***chain*** , associate with Ig binding protein (BiP) and GRP94 and fail to form HL and H2L ***assembly*** intermediates efficiently. The mutations do not irreversibly alter the VH ***domain*** as the small amount of mutant H ***chain*** , which ***assembles*** with L ***chain*** as H2L2, is secreted. The secreted mutant ***Ab*** binds phosphocholine-protein with avidity identical with that of WT ***Ab*** , suggesting that the combining site adopts a WT conformation. A computer-generated model of the PCG1-1 variable region fragment of Ig (Fv) indicates that Ile51 is buried between complementarity-determining region 2 and framework 3 and does not directly contact the L ***chain*** . Thus, the Ile51--> ***Arg*** or Ile51-->Lys mutations impair association with the PCG1-1 L ***chain***

via indirect interactions. These interactions are in part dependent on the nature of the L ***chain*** as the PCG1-1 VH single Ile51--> ***Arg*** or Ile51-->Lys mutants were partially rescued when expressed with the J558L lambda1 L ***chain*** . These results represent the first demonstration that single somatic mutations in V(H) residues can impair Ig secretion and suggest one reason for the conservation of Ile51 in so many Ig VH.

L47 ANSWER 18 OF 132 MEDLINE
ACCESSION NUMBER: 2001184239 MEDLINE
DOCUMENT NUMBER: 21139117 PubMed ID: 11245489
TITLE: Up-regulation of vascular endothelial growth factor in breast cancer cells by the heregulin-beta1-activated p38 signaling pathway enhances endothelial cell migration.
AUTHOR: Xiong S; Grijalva R; Zhang L; Nguyen N T; Pisters P W; Pollock R E; Yu D
CORPORATE SOURCE: Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston 77030, USA.
CONTRACT NUMBER: P30-CA16672 (NCI)
RRO1-CA60448 (NCRR)
SOURCE: CANCER RESEARCH, (2001 Feb 15) 61 (4) 1727-32.
Journal code: 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010329
AB Heregulin (HRG) belongs to a family of polypeptide growth factors that bind to receptor tyrosine kinases ErbB3 and ErbB4. HRG binding induces ErbB3 and ErbB4 ***heterodimerization*** with ErbB2, activating downstream signal transduction. Vascular endothelial growth factor (VEGF) is a primary regulator of physiological angiogenesis and is a major mediator of pathological angiogenesis, such as tumor-associated neovascularization. In this study, we demonstrate that HRG-beta1 increased secretion of VEGF from breast cancer cells in a time- and dosage-dependent manner and that this increase resulted from up-regulation of VEGF mRNA expression via transcriptional activation of the VEGF promoter. Deletion and mutational analysis revealed that a CA-rich upstream HRG response element located between nucleotide-2249 and -2242 in the VEGF promoter mediated HRG-induced transcriptional up-regulation of VEGF. While investigating the downstream signaling pathways involved in HRG-mediated up-regulation of VEGF, we found that HRG activated extracellular signal-regulated protein kinases, Akt kinase, and p38 mitogen-activated protein kinase (MAPK). However, only the specific inhibitor of p38 MAPK (SB203580), not extracellular signal-regulated kinase inhibitor PD98059 nor the inhibitor of phosphatidylinositol 3-kinase-Akt pathway (Wortmannin), blocked the up-regulation of VEGF by HRG. The HRG-stimulated secretion of VEGF from breast cancer cells resulted in increased migration of murine lung endothelial cells, an activity that was inhibited by either VEGF-neutralizing ***antibody*** or SB203580. These results show that HRG can activate p38 MAPK to enhance VEGF transcription via an upstream HRG response element, leading to increased VEGF secretion and angiogenic response in breast cancer cells.

L47 ANSWER 19 OF 132 MEDLINE
ACCESSION NUMBER: 2002048632 MEDLINE
DOCUMENT NUMBER: 21632034 PubMed ID: 11776310
TITLE: A naturally occurring point mutation in the beta3 integrin MIDAS-like domain affects differently alphavbeta3 and alphaIIbbeta3 receptor function.
AUTHOR: Morel-Kopp M C; Melchior C; Chen P; Ammerlaan W; Lecompte T; Kaplan C; Kieffer N
CORPORATE SOURCE: Laboratoire Franco-Luxembourgeois de Recherche Biomedicale (CNRS/CRP-Sante), Centre Universitaire, Luxembourg.

ligand-binding behaviour of the fusion protein was investigated both by solid phase ELISA and in fluorescence titration experiments. Our results demonstrate that the novel fusion protein has retained both ligand specificities. Up to now, dimerized ligand-binding proteins were mostly derived from ***recombinant*** ***antibody*** fragments. Compared with those constructs the duocalins, either with ***bispecific*** or with bivalent target recognition properties, should provide useful reagents for various purposes in biotechnology.

L47 ANSWER 21 OF 132 MEDLINE
ACCESSION NUMBER: 2001385218 MEDLINE
DOCUMENT NUMBER: 21332567 PubMed ID: 11439104
TITLE: Green fluorescent protein (GFP) tagged to the cytoplasmic tail of alphaIIb or beta3 allows the expression of a fully functional integrin alphaIIb(beta3): effect of beta3GFP on alphaIIb(beta3) ligand binding.
AUTHOR: Plancon S; Morel-Kopp M C; Schaffner-Reckinger E; Chen P; Kieffer N
CORPORATE SOURCE: Laboratoire Franco-Luxembourgeois de Recherche Biomedicale (CNRS and CRP-Sante), Centre Universitaire, L-1511 Luxembourg, Grand-Duchy of Luxembourg.
SOURCE: BIOCHEMICAL JOURNAL, (2001 Jul 15) 357 (Pt 2) 529-36. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010827
Last Updated on STN: 20010827
Entered Medline: 20010823
AB Using green fluorescent protein (GFP) as an autofluorescent tag, we report the first successful visualization of a beta3 integrin in a living cell. GFP fused in frame to the cytoplasmic tail of either alphaIIb or beta3 allowed normal expression, ***heterodimerization*** , processing and surface exposure of alphaIIbGFPbeta3 and alphaIIb(beta3)GFP receptors in Chinese hamster ovary (CHO) cells. Direct microscopic observation of the autofluorescent cells in suspension following ***antibody*** -induced alphaIIb(beta3) capping revealed an intense autofluorescent cap corresponding to unlabelled immunoclustered GFP-tagged alphaIIb(beta3). GFP-tagged alphaIIbbeta3 receptors mediated fibrinogen-dependent cell adhesion, were readily detectable in focal adhesions of unstained living cells and triggered p125(FAK) tyrosine phosphorylation similar to wild-type alphaIIb(beta3) (where FAK corresponds to focal adhesion kinase). However, GFP tagged to beta3, but not to alphaIIb, induced spontaneous CHO cell aggregation in the presence of soluble fibrinogen, as well as binding of the fibrinogen mimetic monoclonal ***antibody*** PAC1 in the absence of alphaIIb(beta3) receptor activation. Time-lapse imaging of living transfectants revealed a characteristic redistribution of GFP-tagged alphaIIb(beta3) during the early stages of cell attachment and spreading, starting with alphaIIb(beta3) clustering at the rim of the cell contact area, that gradually overlapped with the boundary of the attached cell, and, with the onset of cell spreading, to a reorganization of alphaIIb(beta3) in focal adhesions. Taken together, our results demonstrate that (1) fusion of GFP to the cytoplasmic tail of either alphaIIb or beta3 integrin subunits allows normal cell surface expression of a functional receptor, and (2) structural modification of the beta3 integrin cytoplasmic tail, rather than the alphaIIb subunit, plays a major role in alphaIIb(beta3) affinity modulation. With the successful direct visualization of functional alphaIIb(beta3) receptors in living cells, the generation of autofluorescent integrins in transgenic animals will become possible, allowing new approaches to study the dynamics of integrin functions.

L47 ANSWER 22 OF 132 MEDLINE
ACCESSION NUMBER: 2002063694 MEDLINE
DOCUMENT NUMBER: 21647525 PubMed ID: 11789029

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020403
Last Updated on STN: 20020522
Entered Medline: 20020521

AB INTRODUCTION: The purpose of this study was to assess the requirement for the RGD sequence of von Willebrand factor (VWF) for its binding to the beta3 chain of integrins and the structural basis for the specificity of monoclonal ***antibody*** (***MoAb***) 9 which specifically binds to VWF and inhibits this interaction. MATERIAL AND METHODS:: Seven point mutations were introduced into VWF by ***site*** -directed ***mutagenesis*** . Mutated recombinant VWF were tested for their multimeric pattern and their ability to bind to purified GPIIb/IIIa, thrombin-activated platelets and ***MoAb*** 9. RESULTS: All recombinant VWF were fully ***multimerized*** . The conservative ***Arg*** 1744 to Lys substitution into the RGD sequence resulted in an 80% loss of function whereas the ***Arg*** to Ala change led to a total loss of function. The two substitutions however did not impair the binding to ***MoAb*** 9. In contrast ***Arg*** 1715 to Ala substitution had no effect on the binding to GPIIb/IIIa but the binding of the corresponding mutated recombinant VWF to ***MoAb*** 9 was strikingly decreased. CONCLUSION: Direct evidence of the role of the structure and the charge of ***Arg*** 1744 into the RGD sequence were established by changing ***Arg*** to Lys (KGD) and to Ala (AGD). Our results also demonstrate that ***Arg*** 1715 is not essential in the function but it is necessary for maintaining the conformation recognized by ***MoAb*** 9 specific for the GPIIb/IIIa-binding ***domain*** of VWF.

L47 ANSWER 27 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:775265 CAPLUS
DOCUMENT NUMBER: 136:132090
TITLE: Investigation of differentially expressed genes during the development of mouse cerebellum
AUTHOR(S): Kagami, Yoshihiro; Furuichi, Teiichi
CORPORATE SOURCE: Laboratory for Molecular Neurogenesis, Brain Science Institute, RIKEN, Wako, 351-0198, Japan
SOURCE: Gene Expression Patterns (2001), 1(1), 39-59
CODEN: GEPEAD; ISSN: 1567-133X
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Before the discovery of DNA microarray and DNA chip technol., the expression of only a small no. of genes could be analyzed at a time. Currently, such technol. allows us the simultaneous anal. of a large no. of genes to systematically monitor their expression patterns that may be assocd. with various biol. phenomena. We utilized the Affymetrix GeneChip Mu11K to analyze the gene expression profile in developing mouse cerebellum to assist in the understanding of the genetic basis of cerebellar development in mice. Our anal. showed 81.6% (10.321/12.654) of the genes represented on the GeneChip were expressed in the postnatal cerebellum, and among those, 8.7% (897/10.321) were differentially expressed with more than a two-fold change in their max. and min. expression levels during the developmental time course. Further anal. of the differentially expressed genes that were clustered in terms of their expression patterns and the function of their encoded products revealed an aspect of the genetic foundation that lies beneath the cellular events and neural network formation that takes place during the development of the mouse cerebellum.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L47 ANSWER 28 OF 132 MEDLINE
ACCESSION NUMBER: 2001195084 MEDLINE
DOCUMENT NUMBER: 21092781 PubMed ID: 11178955

antibodies, monospecific and ***bispecific*** diabodies, and a ***bispecific*** tetravalent ***antibody*** (BiTAb) molecule directed against the CD80 and/or CD86 costimulatory molecules. These ***recombinant*** ***Ab*** molecules were expressed in Escherichia coli, followed by purification and evaluation for specific interaction with their respective antigen in an enzyme-linked immunosorbent assay (ELISA). A specific sandwich ELISA confirmed the bispecificity of the ***bispecific*** diabodies and the BiTAb.

L47 ANSWER 30 OF 132 MEDLINE
ACCESSION NUMBER: 2001098537 MEDLINE
DOCUMENT NUMBER: 20568305 PubMed ID: 10984492
TITLE: New structural and functional aspects of the type I interferon-receptor interaction revealed by comprehensive mutational analysis of the binding ***interface*** .
AUTHOR: Piehler J; Roisman L C; Schreiber G
CORPORATE SOURCE: Department of Biological Chemistry, Weizmann Institute of Science, 76100 Rehovot, Israel.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Dec 22) 275 (51) 40425-33.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010201
AB Type I interferons bind to two cell surface receptors, ifnar1 and ifnar2, as the first step in the activation of several signal transduction pathways that elicit an anti-viral state and an anti-proliferative response. Here, we quantitatively mapped the complete binding region of ifnar2 on interferon (IFN)alpha2 by 35 individual mutations to alanine and isosteric residues. Of the six "hot-spot" residues identified (***Leu*** -30, ***Arg*** -33, ***Arg*** -144, Ala-145, Met-148, and ***Arg*** -149), four are located on the E-helix, which is located at the center of the binding ***site*** flanked by residues on the A-helix and the ***AB*** -loop. The contribution of residues of the D-helix, which have been previously implicated in binding, proved to be marginal for the interaction with the extracellular ***domain*** of ifnar2. Interestingly, the ifnar2 binding ***site*** overlaps the largest continuous hydrophobic patch on IFNalpha2. Thus, hydrophobic interactions seem to play a significant role stabilizing this interaction, with the charged residues contributing toward the rapid association of the complex. Relating the anti-viral and anti-proliferative activity of the various interferon mutants with their affinity toward ifnar2 results in linear function over the whole range of affinities investigated, suggesting that ifnar2 binding is the rate-determining step in cellular activation. Dose-time analysis of the anti-viral response revealed that shortening the incubation time of low-level activation cannot be compensated by higher IFN doses. Considering the strict dependence of the cellular response on affinity, these results suggest that for maintaining transcription of IFN-responsive genes over a longer time period, low but continuous signaling through the IFN receptor is essential.

L47 ANSWER 31 OF 132 MEDLINE
ACCESSION NUMBER: 2000396690 MEDLINE
DOCUMENT NUMBER: 20347252 PubMed ID: 10779520
TITLE: Interferon-alpha induces nmi-IFP35 heterodimeric complex formation that is affected by the phosphorylation of IFP35.
AUTHOR: Zhou X; Liao J; Meyerdierks A; Feng L; Naumovski L; Bottger E C; Omary M B
CORPORATE SOURCE: Palo Alto Veterans Affairs Medical Center and Stanford University, Palo Alto, California 94304, USA.
CONTRACT NUMBER: DK07056 (NIDDK)

DK47918 (NIDDK)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jul 14) 275 (28) 21364-71.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000824
Last Updated on STN: 20000824
Entered Medline: 20000816
AB Nmi and IFP35 are interferon (IFN)-induced proteins. In cells treated with IFN-gamma, Nmi enhances the association of transcription co-activator CBP/p300 with signal transducer and activator of transcription proteins, and IFP35 forms a high molecular weight cytosolic complex of unknown constituents. Here we show that Nmi and IFP35 co-immunoprecipitate with an anti-keratin 19 ***antibody*** , which is due to cross-reaction of the ***antibody*** with Nmi, and suggests an Nmi-IFP35 physical association. In support of this, Nmi and IFP35 co-immunoprecipitate using anti-Nmi and anti-IFP35 antibodies, manifest enhanced colocalization as determined by immunofluorescence staining of IFN-treated cells, and form heterodimers as determined by chemical cross-linking. Nmi and IFP35 are primarily cytosolic proteins, and their interaction is increased after IFN-alpha treatment of cells as early as 1 h after exposure. Sucrose gradient sedimentation and size fractionation showed a shift of Nmi-IFP35 heterodimers toward a heavier fraction (100-200 kDa) in IFN-alpha-treated cells. This dynamic complex formation is reversed by pretreatment with okadaic acid. Two-dimensional gel analysis indicates that the IFN-induced complex formation correlates with IFP35 dephosphorylation. Our data demonstrate Nmi-IFP35 cytosolic localization and ***heterodimerization*** , and an IFN-alpha-regulated molecular event in which Nmi and IFP35 participate, reversibly and by a dephosphorylation dependent fashion, in a 100-200-kDa molecular complex formation.

L47 ANSWER 32 OF 132 MEDLINE
ACCESSION NUMBER: 2001116710 MEDLINE
DOCUMENT NUMBER: 20573560 PubMed ID: 11123892
TITLE: Analysis of ***antibody*** A6 binding to the extracellular interferon gamma receptor alpha-chain by alanine-scanning ***mutagenesis*** and random ***mutagenesis*** with phage display.
AUTHOR: Lang S; Xu J; Stuart F; Thomas R M; Vrijbloed J W; Robinson J A
CORPORATE SOURCE: Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.
SOURCE: BIOCHEMISTRY, (2000 Dec 26) 39 (51) 15674-85.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010215
AB The monoclonal ***antibody*** A6 binds a conformational epitope comprising mainly the CC' surface loop on the N-terminal fibronectin type-III ***domain*** of the extracellular interferon gamma receptor (IFNgammaR). The crystal structure of an A6 Fab-IFNgammaR complex revealed an ***interface*** rich in the aromatic side chains of ***Trp*** , ***Tyr*** , and His residues. These aromatic side chains appear to interact with both polar and hydrophobic groups at the ***interface*** , a property which, in general, may be advantageous for ligand binding. To analyze these interactions in more detail, the affinities of 19 A6 alanine-scanning mutants for the IFNgammaR have been measured, using engineered A6 single chain variable region fragments, and a surface

plasmon resonance biosensor. Energetically important side chains (DeltaG(mutant) - DeltaG(wt) > 2.4 kcal/mol), that form distinct hot spots in the binding ***interface*** , have been identified on both proteins. These include V(L)W92 in A6, whose benzenoid ring appears well situated for a pi-cation (or pi-amine) interaction with the side chain of receptor residue K47 and simultaneously for T-stacking onto the indole ring of W82 in the receptor. At another ***site*** , energetically important residues V(H)W52 and V(H)W53, as well as V(H)D54 and V(H)D56, surround the aliphatic side chain of the hot receptor residue K52. Taken together, the results show that side chains distributed across the ***interface*** , including many aromatic ones, make key energetic contributions to binding. In addition, the receptor CC' loop has been subjected to random ***mutagenesis*** , and receptor mutants with high affinity for A6 have been selected by phage display. Residues previously identified as important for receptor binding to A6 were conserved in the clones isolated. Some mutants, however, showed a much improved affinity for A6, due to changes at Glu55, a residue that appeared to be energetically unimportant for binding the ***antibody*** by alanine-scanning ***mutagenesis*** . An E55P receptor mutant bound A6 with a 600-fold increase in affinity (K(D) approximately 20 pM), which is one of the largest improvements in affinity from a single point mutation reported so far at any protein-protein ***interface*** .

L47 ANSWER 33 OF 132 MEDLINE
ACCESSION NUMBER: 2000270257 MEDLINE
DOCUMENT NUMBER: 20270257 PubMed ID: 10809777
TITLE: Cystine knot mutations affect the folding of the glycoprotein hormone alpha-subunit. Differential secretion and ***assembly*** of partially folded intermediates.
AUTHOR: Darling R J; Ruddon R W; Perini F; Bedows E
CORPORATE SOURCE: Eppley Institute for Research in Cancer and Allied Diseases and the Department of Pharmacology, University of Nebraska Medical Center, Omaha, Nebraska 68198, USA.
CONTRACT NUMBER: CA32949 (NCI)
P 30 CA36727 (NCI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 May 19) 275 (20) 15413-21.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000629
Last Updated on STN: 20000629
Entered Medline: 20000621
AB The common glycoprotein hormone alpha-subunit (GPH-alpha) contains five intramolecular disulfide bonds, three of which form a cystine knot motif (10-60, 28-82, and 32-84). By converting each pair of ***cysteine*** residues of a given disulfide bond to ***alanine*** , we have studied the role of individual disulfide bonds in GPH-alpha folding and have related folding ability to secretion and ***assembly*** with the human chorionic gonadotropin beta-subunit (hCG-beta). Mutation of non-cystine knot disulfide bond 7-31, bond 59-87, or both (leaving only the cystine knot) resulted in an efficiently secreted folding form that was indistinguishable from wild type. Conversely, the cystine knot mutants were inefficiently secreted (<25%). Furthermore, mutation of the cystine knot disulfide bonds resulted in multiple folding intermediates containing 1, 2, or 4 disulfide bonds. High performance liquid chromatographic separation of intracellular and secreted forms of the folding intermediates demonstrated that the most folded forms were preferentially secreted and combined with hCG-beta. From these studies we conclude that: (i) the cystine knot of GPH-alpha is necessary and sufficient for folding and (ii) there is a direct correlation between the extent of GPH-alpha folding, its ability to be secreted, and its ability to ***heterodimerize*** with hCG-beta.

L47 ANSWER 34 OF 132 MEDLINE
ACCESSION NUMBER: 2001091148 MEDLINE
DOCUMENT NUMBER: 20570946 PubMed ID: 11120833
TITLE: Fab chains as an efficient ***heterodimerization*** scaffold for the production of ***recombinant*** bispecific and trispecific ***antibody*** derivatives.
AUTHOR: Schoonjans R; Willems A; Schoonooghe S; Fiers W; Grooten J; Mertens N
CORPORATE SOURCE: Molecular Immunology Unit, Department of Molecular Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, Ghent, Belgium.
SOURCE: JOURNAL OF IMMUNOLOGY, (2000 Dec 15) 165 (12) 7050-7. Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125
AB Due to their multispecificity and versatility, bispecific Abs (BsAbs) are promising therapeutic tools in tomorrow's medicine. Especially intermediate-sized BsAbs that combine body retention with tissue penetration are valuable for therapy but necessitate expression systems that favor ***heterodimerization*** of the binding sites for large-scale application. To identify ***heterodimerization*** domains to which single-chain variable fragments (scFv) can be fused, we compared the efficiency of ***heterodimerization*** of CL and CH1 constant domains with complete L and Fd chains in mammalian cells. We found that the isolated CL:CH1 domain interaction was inefficient for secretion of heterodimers. However, when the complete L and Fd chains were used, secretion of L:Fd heterodimers was highly successful. Because these Fab chains contribute a binding moiety, C-terminal fusion of a scFv molecule to the L and/or Fd chains generated BsAbs or trispecific Abs (TsAbs) of intermediate size (75-100 kDa). These disulfide-stabilized bispecific Fab-scFv ("bibody") and trispecific Fab-(scFv)(2) ("tribody") heterodimers represent up to 90% of all secreted ***Ab*** fragments in the mammalian expression system and possess fully functional binding moieties. Furthermore, both molecules recruit and activate T cells in a tumor cell-dependent way, whereby the trispecific derivative can exert this activity to two different tumor cells. Thus we propose the use of the disulfide-stabilized L:Fd heterodimer as an efficient platform for production of intermediate-sized BsAbs and TsAbs in mammalian expression systems.

L47 ANSWER 35 OF 132 MEDLINE
ACCESSION NUMBER: 2000439316 MEDLINE
DOCUMENT NUMBER: 20396331 PubMed ID: 10938130
TITLE: In-depth mutational analysis of the promyelocytic leukemia zinc finger BTB/POZ ***domain*** reveals motifs and residues required for biological and transcriptional functions.
AUTHOR: Melnick A; Ahmad K F; Arai S; Polinger A; Ball H; Borden K L; Carlile G W; Prive G G; Licht J D
CORPORATE SOURCE: Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029, USA.
CONTRACT NUMBER: CA59936 (NCI)
KO8 CA73762 (NCI)
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (2000 Sep) 20 (17) 6550-67. Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000928

Last Updated on STN: 20000928
Entered Medline: 20000921

AB The promyelocytic leukemia zinc finger (PLZF) protein is a transcription factor disrupted in patients with t(11;17)(q23;q21)-associated acute promyelocytic leukemia. PLZF contains an N-terminal BTB/POZ ***domain*** which is required for dimerization, transcriptional repression, formation of high-molecular-weight DNA-protein complexes, nuclear sublocalization, and growth suppression. X-ray crystallographic data show that the PLZF BTB/POZ ***domain*** forms an obligate homodimer via an extensive ***interface*** . In addition, the dimer possesses several highly conserved features, including a charged pocket, a hydrophobic monomer core, an exposed hydrophobic surface on the floor of the dimer, and two negatively charged surface patches. To determine the role of these structures, mutational analysis of the BTB/POZ ***domain*** was performed. We found that point mutations in conserved residues that disrupt the dimer ***interface*** or the monomer core result in a misfolded nonfunctional protein. Mutation of key residues from the exposed hydrophobic surface suggests that these are also important for the stability of PLZF complexes. The integrity of the charged-pocket region was crucial for proper folding of the BTB/POZ ***domain*** . In addition, the pocket was critical for the ability of the BTB/POZ ***domain*** to repress transcription. Alteration of charged-pocket residue ***arginine*** 49 to a glutamine (mutant R49Q) yields a ***domain*** that can still dimerize but activates rather than represses transcription. In the context of full-length PLZF, a properly folded BTB/POZ ***domain*** was required for all PLZF functions. However, PLZF with the single pocket mutation R49Q repressed transcription, while the double mutant D35N/R49Q could not, despite its ability to dimerize. These results indicate that PLZF requires the BTB/POZ ***domain*** for dimerization and the charged pocket for transcriptional repression.

L47 ANSWER 36 OF 132 MEDLINE
ACCESSION NUMBER: 2000290813 MEDLINE
DOCUMENT NUMBER: 20290813 PubMed ID: 10828942
TITLE: Three-dimensional structures of the free and antigen-bound Fab from monoclonal antilysozyme ***antibody*** HyHEL-63(,).
AUTHOR: Li Y; Li H; Smith-Gill S J; Mariuzza R A
CORPORATE SOURCE: Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, Maryland 20850, USA.
CONTRACT NUMBER: GM5280 (NIGMS)
SOURCE: BIOCHEMISTRY, (2000 May 30) 39 (21) 6296-309.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1DQJ; PDB-1DQM; PDB-1DQQ
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000720
Last Updated on STN: 20000720
Entered Medline: 20000711

AB Antigen- ***antibody*** complexes provide useful models for studying the structure and energetics of protein-protein interactions. We report the cloning, bacterial expression, and crystallization of the antigen-binding fragment (Fab) of the anti-hen egg white lysozyme (HEL) ***antibody*** HyHEL-63 in both free and antigen-bound forms. The three-dimensional structure of Fab HyHEL-63 complexed with HEL was determined to 2.0 A resolution, while the structure of the unbound ***antibody*** was determined in two crystal forms, to 1.8 and 2.1 A resolution. In the complex, 19 HyHEL-63 residues from all six complementarity-determining regions (CDRs) of the ***antibody*** contact 21 HEL residues from three discontinuous polypeptide segments of the antigen. The ***interface*** also includes 11 bound water molecules, 3 of which are completely buried in the complex. Comparison of the structures of free and bound Fab HyHEL-63 reveals that several of the

ordered water molecules in the free ***antibody*** -combining site are retained and that additional waters are added upon complex formation. The ***interface*** waters serve to increase shape and chemical complementarity by filling cavities between the interacting surfaces and by contributing to the hydrogen bonding network linking the antigen and ***antibody*** . Complementarity is further enhanced by small (<3 A) movements in the polypeptide backbones of certain ***antibody*** CDR loops, by rearrangements of side chains in the ***interface*** , and by a slight shift in the relative orientation of the V(L) and V(H) domains. The combining site residues of complexed Fab HyHEL-63 exhibit reduced temperature factors compared with those of the free Fab, suggesting a loss in conformational entropy upon binding. To probe the relative contribution of individual antigen residues to complex stabilization, single alanine substitutions were introduced in the epitope of HEL recognized by HyHEL-63, and their effects on ***antibody*** affinity were measured using surface plasmon resonance. In agreement with the crystal structure, HEL residues at the center of the ***interface*** that are buried in the complex contribute most to the binding energetics (DeltaG(mutant) - DeltaG(wild type) > 3.0 kcal/mol), whereas the apparent contributions of solvent-accessible residues at the periphery are much less pronounced (<1.5 kcal/mol). In the latter case, the mutations may be partially compensated by local rearrangements in solvent structure that help preserve shape complementarity and the ***interface*** hydrogen bonding network.

L47 ANSWER 37 OF 132 MEDLINE
ACCESSION NUMBER: 2000485702 MEDLINE
DOCUMENT NUMBER: 20487175 PubMed ID: 11034362
TITLE: Mutant mouse lysozyme carrying a minimal T cell epitope of hen egg lysozyme evokes high autoantibody response.
AUTHOR: Tsujihata Y; So T; Chijiiwa Y; Hashimoto Y; Hirata M; Ueda T; Imoto T
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan.
SOURCE: JOURNAL OF IMMUNOLOGY, (2000 Oct 1) 165 (7) 3606-11. Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001103

AB Self proteins including foreign T cell epitope induce autoantibodies. We evaluated the relationship between the size of foreign Ag introduced into self protein and the magnitude of autoantibody production. Mouse lysozyme (ML) was used as a model self protein, and we prepared three different ML derivatives carrying T cell epitope of hen egg white lysozyme (HEL) 107-116, i.e, heterodimer of ML and HEL (ML-HEL), chimeric lysozyme that has residue 1-82 of ML and residue 83-130 of HEL in its sequence (chiMH), and mutant ML that has triple mutations rendering the most potent T cell epitope of HEL (sequence 107-116). Immunization of BALB/c mice with these three ML derivatives induced anti-ML autoantibody responses, whereas native ML induced no detectable response. In particular, mutML generated a 10(4) times higher autoantibody titer than did ML-HEL. Anti-HEL107-116 T cell-priming activities were almost similar among the ML derivatives. The ***heterodimerization*** of mutant ML and HEL led to significant reduction of the autoantibody response, whereas the mixture did not. These results show that size of the nonself region in modified self Ag has an important role in determining the magnitude of the autoantibody response, and that decrease in the foreign region in a modified self protein may cause high-titered autoantibody response.

L47 ANSWER 38 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000079721 EMBASE
TITLE: Identification of contact residues and definition of the

CAR-binding ***site*** of adenovirus type 5 fiber protein.
AUTHOR: Kirby I.; Davison E.; Beavil A.J.; Soh C.P.C.; Wickham T.J.; Roelvink P.W.; Kovesdi I.; Sutfon B.J.; Santis G.
CORPORATE SOURCE: G. Santis, Dept. of Resp. Med. and Allergy, Thomas Guy House, Guy's Hospital, St. Thomas St., London SE1 9RT, United Kingdom. george.santis@kcl.ac.uk
SOURCE: Journal of Virology, (2000) 74/6 (2804-2813).
Refs: 34
ISSN: 0022-538X CODEN: JOVIAM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The binding of adenovirus (Ad) fiber knob to its cellular receptor, the coxsackievirus and Ad receptor (CAR), promotes virus attachment to cells and is a major determinant of Ad tropism. Analysis of the kinetics of binding of Ad type 5 (Ad5) fiber knob to the soluble extracellular domains of CAR together (sCAR) and each immunoglobulin (Ig) ***domain*** (IgV and IgC2) independently by surface plasmon resonance demonstrated that the IgV ***domain*** is necessary and sufficient for binding, and no additional membrane components are required to confer high-affinity binding to Ad5 fiber knob. Four Ad5 fiber knob mutations, Ser408Glu and Pro409Lys in the ***AB*** loop, Tyr477Ala in the DG loop, and Leu485Lys in .beta. strand F, effectively abolished high-affinity binding to CAR, while Ala406Lys and Arg412Asp in the ***AB*** loop and Arg481Glu in .beta. strand E significantly reduced the level of binding. Circular dichroism spectroscopy showed that these mutations do not disorder the secondary structure of the protein, implicating Ser408, Pro409, Tyr477, and Leu485 as contact residues, with Ala406, Arg412, and Arg481 being peripherally or indirectly involved in CAR binding. The critical residues have exposed side chains that form a patch on the surface, which thus defines the high-affinity ***interface*** for CAR. Additional ***site*** -directed ***mutagenesis*** of Ad5 fiber knob suggests that the binding ***site*** does not extend to the adjacent subunit or toward the edge of the R sheet. These findings have implications for our understanding of the biology of Ad infection, the development of novel Ad vectors for targeted gene therapy, and the construction of peptide inhibitors of Ad infection.

L47 ANSWER 39 OF 132 MEDLINE
ACCESSION NUMBER: 2000150127 MEDLINE
DOCUMENT NUMBER: 20150127 PubMed ID: 10684865
TITLE: Inhibition of interleukin 7 receptor signaling by antigen receptor ***assembly*** .
AUTHOR: Smart F M; Venkitaraman A R
CORPORATE SOURCE: Wellcome Trust Centre for the Study of Molecular Mechanisms in Disease, and the Cancer Research Campaign Department of Oncology, University of Cambridge, The Cambridge Institute for Medical Research, Cambridge CB2 2XY, United Kingdom.
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (2000 Feb 21) 191 (4) 737-42.
Journal code: 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000419
AB After the productive rearrangement of immunoglobulin (Ig) ***heavy*** ***chain*** genes, precursor (pre-)B lymphocytes undergo a limited number of cell divisions in response to interleukin (IL)-7. Here, we present evidence that this phase of IL-7-dependent expansion is constrained by an inhibitory signal initiated by antigen receptor

assembly . A line of pre-B cells from normal murine bone marrow that expresses a mu ***heavy*** ***chain*** with a D-proximal V(H)7183.2 region divides continuously in IL-7. IL-7 responsiveness ceases upon differentiation to the mu(1), kappa(1) stage, despite continuing expression of the IL-7 receptor (IL-7R), suggesting that antigen receptor ***assembly*** inhibits IL-7 responsiveness. This is confirmed by introduction of a rearranged lambda ***light*** ***chain*** gene, which inhibits proliferative signaling through the IL-7R. Inhibition is specific to the IL-7R, because it is overcome by replacement of the IL-7R cytoplasmic ***domain*** with corresponding sequences from the closely related IL-2Rbeta ***chain*** . Alteration of a single ***tyrosine*** residue, Tyr410, in the IL-7R cytoplasmic ***domain*** to ***phenylalanine*** also prevents the inhibition of proliferation after antigen receptor ***assembly*** . Thus, the loss of IL-7 responsiveness after antigen receptor ***assembly*** may be mediated through the recruitment of an inhibitory molecule to this residue. Our findings identify a novel mechanism that limits cytokine-dependent proliferation during B lymphopoiesis. This mechanism may be essential for the proper regulation of peripheral B lymphocyte numbers.

L47 ANSWER 40 OF 132 MEDLINE
ACCESSION NUMBER: 2001023692 MEDLINE
DOCUMENT NUMBER: 20453810 PubMed ID: 10995872
TITLE: Heregulin regulates the actin cytoskeleton and promotes invasive properties in breast cancer cell lines.
AUTHOR: Hijazi M M; Thompson E W; Tang C; Coopman P; Torri J A; Yang D; Mueller S C; Lupu R
CORPORATE SOURCE: Vincent T. Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC 20007, USA.
CONTRACT NUMBER: RO1-DK49049 (NIDDK)
SOURCE: INTERNATIONAL JOURNAL OF ONCOLOGY, (2000 Oct) 17 (4) 629-41.
Journal code: 9306042. ISSN: 1019-6439.
PUB. COUNTRY: Greece
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001113

AB The metastatic process requires changes in tumor cell adhesion properties, cell motility and remodeling of the extracellular matrix. The erbB2 proto-oncogene is overexpressed in approximately 30% of breast cancers and is a major prognostic parameter when present in invasive disease. A ligand for the erbB2 receptor has not yet been identified but it can be activated by ***heterodimerization*** with heregulin (HRG)-stimulated erbB3 and erbB4 receptors. The HRGs are a family of polypeptide growth factors that have been shown to play a role in embryogenesis, tumor formation, growth and differentiation of breast cancer cells. The erbB3 and erbB4 receptors are involved in transregulation of erbB2 signaling. The work presented here suggests biological roles for HRG including regulation of the actin cytoskeleton and induction of motility and invasion in breast cancer cells. HRG-expressing breast cancer cell lines are characterized by low erbB receptor levels and a high invasive and metastatic index, while those which overexpress erbB2 demonstrate minimal invasive potential in vitro and are non-tumorigenic in vivo. Treatment of the highly tumorigenic and metastatic HRG-expressing breast cancer cell line MDA-MB-231 with an HRG-neutralizing ***antibody*** significantly inhibited proliferation in culture and motility in the Boyden chamber assay. Addition of exogenous HRG to non-invasive erbB2 overexpressing cells (SKBr-3) at low concentrations induced formation of pseudopodia, enhanced phagocytic activity and increased chemomigration and invasion in the Boyden chamber assay. The specificity of the chemomigration response to HRG is demonstrated by inhibition with the anti-HRG neutralizing ***antibody*** . These results suggest that either HRG can act as an autocrine or paracrine ligand to promote the invasive behavior of breast cancer cells

in vitro or thus may enhance the metastatic process in vivo.

L47 ANSWER 41 OF 132 MEDLINE
ACCESSION NUMBER: 2001077341 MEDLINE
DOCUMENT NUMBER: 20538224 PubMed ID: 11084652
TITLE: Placental defects in ARNT-knockout conceptus correlate with localized decreases in VEGF-R2, Ang-1, and Tie-2.
AUTHOR: Abbott B D; Buckalew A R
CORPORATE SOURCE: Reproductive Toxicology Division, National Health and Environmental Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, North Carolina 27711, USA.. abbott.barbara@epa.gov
SOURCE: DEVELOPMENTAL DYNAMICS, (2000 Dec) 219 (4) 526-38. Journal code: 9201927. ISSN: 1058-8388.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010111
AB The aryl hydrocarbon receptor nuclear translocator (ARNT) is a transcriptional regulator that ***heterodimerizes*** with Per-ARNT-Sim (PAS) proteins. ARNT also dimerizes with hypoxia inducible factor1alpha (HIF1alpha), inducing expression of vascular endothelial cell growth factor (VEGF) to promote angiogenesis. The angiogenesis/vasculogenesis pathway is required for embryonic survival and includes several receptors (VEGFR1, VEGFR2, Tie2) and ligands (VEGF, Ang1, Ang2, neuropillin). Transgenic knockout of ARNT in mice is lethal due to abnormal placentation. This study examines the VEGF pathway in GD9.5 embryos of wild-type (+/+), heterozygous (+/-), or knockout (-/-) ARNT genotype. All genotypes expressed abundant VEGF in trophoblastic giant cells. However, -/- conceptuses had less VEGFR2 in placental labyrinth and trophoblastic giant cells. Ang1 and Tie2 decreased in trophoblastic giant cells and Ang2 was decreased in placental endothelial cells. Abnormal development of the labyrinth correlated with decreased binding of VEGF and decreased expression of VEGFR2. In addition, VEGFR2 seemed to be the primary VEGF binding receptor in the labyrinth and blood lacunae of the placenta, as binding could be eliminated by masking the VEGFR2 receptor with inactive ***antibody*** complex. VEGFR1 may be primarily responsible for binding of VEGF to yolk sac and embryonic tissues, as masking VEGFR2 did not reduce VEGF binding in those areas, and it is interesting that major structural defects were also not found in those regions. In summary, in the ARNT knockout conceptus, the impact of ARNT deficiency on placental expression of VEGFR2 seems to provide an explanation for the failure of the placental labyrinth to progress, whereas the vascularization of the yolk sac and embryo appear relatively unaffected on GD9.5. Published 2000 Wiley-Liss, Inc.

L47 ANSWER 42 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000396022 EMBASE
TITLE: Rat testicular myotubularin, a protein ***tyrosine*** phosphatase expressed by sertoli and germ cells, is a potential marker for studying cell-cell interactions in the rat testis.
AUTHOR: Li J.C.H.; Samy E.T.; Grima J.; Chung S.S.W.; Mruk D.; Lee W.M.; Silvestrini B.; Cheng C.Y.
CORPORATE SOURCE: C.Y. Cheng, Population Council, Center for Biomedical Research, Rockefeller University, 1230 York Avenue, New York, NY 10021, United States.
ycheng@popcbr.rockefeller.edu
SOURCE: Journal of Cellular Physiology, (2000) 185/3 (366-385).
Refs: 90
ISSN: 0021-9541 CODEN: JCLLAX
COUNTRY: United States
DOCUMENT TYPE: Journal; Article

Journal code: 9005353. ISSN: 1043-4666.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000419

AB Oncostatin M (OSM) and leukaemia inhibitory factor (LIF) exhibit pleiotropic biological activities and share many structural and genetic features. The two cytokines bind with high affinity to the same receptor (LIF/OSM receptor), which consists of the LIF receptor alpha chain (LIFRalpha) and the signal transduction unit gp130. A soluble form of the beta chain of the receptor complex called soluble gp130 (sgp130) has been cloned. In this study, we sought to determine whether ***recombinant*** sgp130 or anti-gp130 ***Ab*** could attenuate the resorption of proteoglycans induced by OSM and LIF in articular cartilage explants. The results show that at high concentrations sgp130 is capable of attenuating both LIF and OSM mediated resorption. In contrast, anti-gp130 ***Ab*** selectively inhibited the stimulation of proteoglycan (PG) release by OSM, albeit minimally. The failure of anti-gp130 to attenuate LIF stimulated PG resorption may be due to the normal interaction of LIF with LIFRalpha and unfettered ***heterodimerization*** of LIFRalpha with gp130 in the presence of the ***antibody*** . The results indicate that sgp130 and anti-gp130 can modulate cartilage PG metabolism in vitro. Whether sgp130 may have therapeutic activity in models of arthritis or indeed in arthritic diseases remains to be determined.

L47 ANSWER 45 OF 132 MEDLINE
ACCESSION NUMBER: 2001047122 MEDLINE
DOCUMENT NUMBER: 20397858 PubMed ID: 10943942
TITLE: Using a ***heavy*** chain-loss hybridoma 26.4.1LL for studying the structural basis of immunoglobulin chain association.
AUTHOR: Yang C Y
CORPORATE SOURCE: Institute of Molecular Biology, National Chung Hsing University, Taichung, Taiwan, ROC.
SOURCE: PROCEEDINGS OF THE NATIONAL SCIENCE COUNCIL, REPUBLIC OF CHINA. PART B, LIFE SCIENCES, (2000 Jul) 24 (3) 101-7.
Journal code: 8502426. ISSN: 0255-6596.
PUB. COUNTRY: CHINA (REPUBLIC: 1949-)
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001204

AB One of the mechanisms contributing to ***antibody*** diversity is created by the association of different ***heavy*** and ***light*** chains. The combinability of ***heavy*** and ***light*** chains has been studied previously in two systems: in vitro chain recombination and hybrid hybridoma. Here, a novel in vivo chain combination assay system involving a ***heavy*** chain-loss variant, 26.4.1LL, producing two kappa ***light*** chains (L(DEX) and L(MPC)) different in size is described. In conjunction with DNA transfection, immunoprecipitation and SDS-PAGE, the structural basis of noncovalent interaction between ***heavy*** and ***light*** chains can be elucidated systematically by examining the relative association tendency of a ***heavy*** chain with two ***light*** chains. To demonstrate the usefulness of this system, three stably transfected 26.4.1LL cell lines expressing gamma2b ***heavy*** chains, designated as H(DEX), H(CHI) and H(ARS), respectively, with structural interrelated variable regions were generated: H(DEX) differs from H(CHI) only in framework regions whereas H(CHI) differs from H(ARS) in complementarity-determining regions. The

relative amounts (R values) of L(DEX) and L(MPC) associated with the ***heavy*** chains H(DEX), H(CHI) and H(ARS) in the assembled immunoglobulin molecules were found to be 1.02, 0.64 and 0.05, respectively, suggesting that the complementarity-determining regions and framework regions contribute equally to the V(L)-V(H) interaction. This conclusion is consistent with previous observations based on calculation of the buried area in the V(L)-V(H) ***interface*** , thus demonstrating the usefulness of this system.

L47 ANSWER 46 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:819393 CAPLUS
DOCUMENT NUMBER: 132:45805
TITLE: Monitoring gene expression or protein levels in evaluating an organism's response to drugs of abuse
INVENTOR(S): Miles, Michael F.; Lai, Chao-qiang; Lockhart, David J.
PATENT ASSIGNEE(S): Regents of the University of California, USA
SOURCE: PCT Int. Appl., 98 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9967267	A1	19991229	WO 1999-US13839	19990622
AU 9946963	A1	20000110	AU 1999-46963	19990622

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1998-90268P P 19980622
US 1999-337022 A 19990621
WO 1999-US13839 W 19990622

AB This invention pertains to the identification of genes whose expression levels are altered by chronic exposure of a cell, tissue, or organism to one or more drugs of abuse (e.g. alc., stimulants, opiates, etc.). In one embodiment, this invention provides a method of monitoring the response of a cell to a drug of abuse. The method involves contacting the cell with the drug of abuse; providing a biol. sample comprising the cell; and detecting, in the sample, the expression of one or more genes or ESTs identified herein, where a difference between the expression of one or more of said genes of ESTs in said sample and one or more of said genes or ESTs in a biol. sample not contacted with said drug of abuse indicates a response of the cell to the drug of abuse. Genes and ESTs whose expression was altered by contact of a cell with EtOH were identified by exposing human neuroblastoma cell line SH-SY5Y-AH1861. Four genes showed a dose-dependent response to EtOH and are therefore believed to represent important targets of EtOH: dopamine .beta. hydroxylase, sodium-dependent norepinephrine transporter, delta-like protein, and monocyte chemoattractant peptide 1. Similar studies were conducted by exposing mice to cocaine. Altered gene expression in the hippocampus, ventral tegmental area, prefrontal cortex, and nucleus accumbens were obsd.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L47 ANSWER 47 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:222965 CAPLUS
DOCUMENT NUMBER: 130:251215
TITLE: Catalytic antibodies and a method of producing same
INVENTOR(S): Koentgen, Frank; Suess, Gabriele Maria; Tarlinton, David Mathew; Treutlein, Herbert Rudolf

PATENT ASSIGNEE(S): Amrad Operations Pty. Ltd., Australia
SOURCE: PCT Int. Appl., 101 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9915563	A1	19990401	WO 1998-AU783	19980918
CA 2304365	AA	19990401	CA 1998-2304365	19980918
AU 9891467	A1	19990412	AU 1998-91467	19980918
AU 744911	B2	20020307		
EP 1015494	A1	20000705	EP 1998-943578	19980918
GB 2345694	A1	20000719	GB 2000-9758	19980918
GB 2345694	B2	20020717		

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.: AU 1997-9306 A 19970919
WO 1998-AU783 W 19980918

AB The present invention relates generally to a growth factor precursor and its use to select prodn. of antigen specific catalytic antibodies. Such catalytic antibodies are produced following B cell activation and proliferation induced by catalytic cleavage products of a target antigen portion of the growth factor precursor of the present invention. A particularly useful form of the growth factor precursor is as a nucleic acid vaccine. The nucleic acid vaccine of the present invention preferably further comprises a mol. adjuvant. Another aspect of the present invention comprises a growth factor precursor in multimeric form. The growth factor precursor of the present invention is useful for generating catalytic antibodies for both therapeutic, diagnostic and industrial purposes, esp. for treating rheumatoid arthritis, AIDS and Alzheimer's disease and others. Thus, pASK75 encoding ompA signal sequence and LHL was constructed and expressed in Escherichia coli, and LHL was purified over a human IgG affinity column. Similarly, LHL.seq contg. N-terminal FLAG epitope (DYKDDDDK) and C-terminal strep-tag (AWRHPQFGG) was generated, while the FLAG epitope was added to facilitate the secretion of LHL.seq and strep-tag was added for purifn. by streptavidin column. TLHL comprising FLAG-kappa-linker-tobacco etch virus protease (TEV)-LHL-strep-tag was also generated and CATAB-TEV was assembled from TLHL and kappa by PCR. B cell proliferation and activation, B7-1 expression, MHC class II induction, detection of CATAB-specific catalytic antibodies in serum, OMP-induced ***multimerization*** , design of novel multimeric mitogen, etc. were tested with the prepd.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L47 ANSWER 48 OF 132 MEDLINE
ACCESSION NUMBER: 1999287863 MEDLINE
DOCUMENT NUMBER: 99287863 PubMed ID: 10358015
TITLE: Homo- and ***heterodimerization*** of synapsins.
AUTHOR: Hosaka M; Sudhof T C
CORPORATE SOURCE: Center for Basic Neuroscience and Department of Molecular Genetics, Howard Hughes Medical Institute, The University of Texas Southwestern Medical School, Dallas, Texas 75235, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jun 11) 274 (24)

16747-53.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990715
Last Updated on STN: 19990715
Entered Medline: 19990706

AB In vertebrates, synapsins constitute a family of synaptic vesicle proteins encoded by three genes. Synapsins contain a central ATP-binding domain, the C-domain, that is highly homologous between synapsins and evolutionarily conserved in invertebrates. The crystal structure of the C-domain from synapsin I revealed that it constitutes a large (>300 amino acids), independently folded domain that forms a tight dimer with or without bound ATP. We now show that the C-domains of all synapsins form homodimers, and that in addition, C-domains from different synapsins associate into heterodimers. This conclusion is based on four findings: 1) in yeast two-hybrid screens with full-length synapsin IIa as a bait, the most frequently isolated prey cDNAs encoded the C-domain of synapsins; 2) quantitative yeast two-hybrid protein-protein binding assays demonstrated pairwise strong interactions between all synapsins; 3) immunoprecipitations from transfected COS cells confirmed that synapsin II heteromultimerizes with synapsins I and III in intact cells, and similar results were obtained with bacterial expression systems; and 4) quantification of the synapsin III level in synapsin I/II double knockout mice showed that the level of synapsin III is decreased by 50%, indicating that heteromultimerization of synapsin III with synapsins I or II occurs in vivo and is required for protein stabilization. These data suggest that synapsins coat the surface of synaptic vesicles as homo- and heterodimers in which the C-domains of the various subunits have distinct regulatory properties and are flanked by variable C-terminal sequences. The data also imply that synapsin III does not compensate for the loss of synapsins I and II in the double knockout mice.

L47 ANSWER 49 OF 132 MEDLINE
ACCESSION NUMBER: 2000060993 MEDLINE
DOCUMENT NUMBER: 20060993 PubMed ID: 10595544
TITLE: Production of soluble alphabeta T-cell receptor heterodimers suitable for biophysical analysis of ligand binding.
AUTHOR: Willcox B E; Gao G F; Wyer J R; O'Callaghan C A; Boulter J M; Jones E Y; van der Merwe P A; Bell J I; Jakobsen B K
CORPORATE SOURCE: MRC Human Immunology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom.
SOURCE: PROTEIN SCIENCE, (1999 Nov) 8 (11) 2418-23.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000107

AB A method to produce alphabeta T-cell receptors (TCRs) in a soluble form suitable for biophysical analysis was devised involving in vitro refolding of a TCR fusion protein. Polypeptides corresponding to the variable and constant domains of each chain of a human and a murine receptor, fused to a coiled coil ***heterodimerization*** motif from either c-Jun (alpha) or v-Fos (beta), were overexpressed separately in Escherichia coli. Following recovery from inclusion bodies, the two chains of each receptor were denatured, and then refolded together in the presence of denaturants. For the human receptor, which is specific for the immunodominant influenza A HLA-A2-restricted matrix epitope (M58-66), a heterodimeric protein was purified in milligram yields and found to be homogeneous, monomeric,

antibody -reactive, and stable at concentrations lower than 1 microM. Using similar procedures, analogous results were obtained with a murine receptor specific for an influenza nucleoprotein epitope (366-374) restricted by H2-Db. Production of these receptors has facilitated a detailed analysis of viral peptide-Major Histocompatibility Complex (peptide-MHC) engagement by the TCR using both surface plasmon resonance (SPR) and, in the case of the human TCR, isothermal titration calorimetry (ITC) (Willcox et al., 1999). The ***recombinant*** methods described should enable a wide range of TCR-peptide-MHC interactions to be studied and may also have implications for the production of other heterodimeric receptor molecules.

L47 ANSWER 50 OF 132 MEDLINE
ACCESSION NUMBER: 1999126532 MEDLINE
DOCUMENT NUMBER: 99126532 PubMed ID: 9925781
TITLE: Folding and assembly of an ***antibody*** Fv fragment, a heterodimer stabilized by antigen.
AUTHOR: Jager M; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut der Universitat Zurich, Winterthurerstr. 190, Zurich, CH-8057, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1999 Feb 5) 285 (5) 2005-19. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990402
Last Updated on STN: 19990402
Entered Medline: 19990325
AB The folding and assembly of the Fv fragment of the phosphorylcholine binding ***antibody*** McPC603, a non-covalent heterodimer of the variable domains VH and VL, was investigated. Since both domains, each engineered for stability and folding efficiency, could now be obtained in native and soluble form by themselves, fluorescence spectra of VH and VL in unfolded, folded and associated states can be reported. VH and VL only associate when they are native, and the stability of the heterodimer is strongly increased in the presence of antigen. VH rapidly folds into an hyperfluorescent intermediate, and the native state is reached in two parallel, proline-independent reactions. VL displays two fast refolding reactions, which are followed by two slower phases, limited by proline cis/trans-isomerization. The rate-limiting step for both the Fv and the scFv (single-chain Fv) fragment is the formation of the native VH-VL ***interface*** , which depends on ProL95 being in cis. The folding of the Fv fragment is fast after short-term denaturation or in the presence of proline cis/trans-isomerase catalysis, but the scFv fragment falls into a kinetic trap, observed by the persistence of the slow phases under all conditions. Furthermore, the scFv fragment, but not the Fv fragment, gives rise to premature ***interface*** formation, indicated by the fluorescence spectra and a much higher transient binding of 8-anilino-1-naphthalene sulfonate. The analysis of the folding pathway of the domains VH and VL in isolation and in non-covalent and covalent assemblies should provide helpful insights into the folding of multimeric proteins in general, and for the further engineering of stable and well-folding ***antibody*** fragments in particular.

L47 ANSWER 51 OF 132 MEDLINE
ACCESSION NUMBER: 2000036334 MEDLINE
DOCUMENT NUMBER: 20036334 PubMed ID: 10567237
TITLE: Fine mapping of inhibitory anti-alpha5 monoclonal ***antibody*** epitopes that differentially affect integrin-ligand binding.
AUTHOR: Burrows L; Clark K; Mould A P; Humphries M J
CORPORATE SOURCE: Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, U.K.

SOURCE: BIOCHEMICAL JOURNAL, (1999 Dec 1) 344 Pt 2 527-33.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20000229
Entered Medline: 20000211
AB The high-affinity interaction of integrin alpha5beta1 with the central cell-binding ***domain*** of fibronectin requires both the ***Arg*** -Gly-Asp (RGD) sequence (in the tenth type III repeat) and a second ***site*** Pro-His-Ser- ***Arg*** -Asn (PHSRN) in the adjacent ninth type III repeat, which synergizes with RGD. ***Arg*** - ***Arg*** -Glu-Thr-Ala- ***Trp*** -Ala (RRETAWA) is a novel peptidic ligand for alpha5beta1, identified by phage display, which blocks alpha5beta1-mediated cell adhesion to fibronectin. A key question is the location of the binding sites for these ligand sequences within the integrin. In this study we have identified residues that form part of the epitopes of three inhibitory anti-alpha5 monoclonal antibodies (mAbs): 16, P1D6 and SNAKA52. These mAbs have distinct functional properties. ***mAb*** 16 blocks the recognition of RGD and RRETAWA, whereas P1D6 blocks binding to the synergy sequence. The binding of SNAKA52 is inhibited by anti-beta1 mAbs, indicating that its epitope is close to the ***interface*** between the alpha and beta subunits. Residues in human alpha5 were replaced with the corresponding residues in mouse alpha5 by ***site*** -directed ***mutagenesis*** ; wild-type or mutant human alpha5 was expressed on the surface of alpha5-deficient Chinese hamster ovary cells. ***mAb*** binding was assessed by flow cytometry and by adhesion to the central cell-binding ***domain*** of fibronectin or RRETAWA by cell attachment assay. All three epitopes were located to different putative loops in the N-terminal ***domain*** of alpha5. As expected, disruption of these epitopes had no effect on ligand recognition by alpha5beta1. The locations of these epitopes are consistent with the beta-propeller model for integrin alpha-subunit structure and allow us to propose a topological image of the integrin-ligand complex.

L47 ANSWER 52 OF 132 MEDLINE
ACCESSION NUMBER: 1999124377 MEDLINE
DOCUMENT NUMBER: 99124377 PubMed ID: 9927187
TITLE: Regulation of cyclooxygenase-2 pathway by HER2 receptor.
AUTHOR: Vadlamudi R; Mandal M; Adam L; Steinbach G; Mendelsohn J; Kumar R
CORPORATE SOURCE: Department of Clinical Investigation, The University of Texas MD Anderson Cancer Center, Houston 77030, USA.
CONTRACT NUMBER: CA65746 (NCI)
SOURCE: ONCOGENE, (1999 Jan 14) 18 (2) 305-14.
Journal code: 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990301
Last Updated on STN: 20000303
Entered Medline: 19990216
AB Emerging lines of evidence suggest that in addition to growth factors, the process of colorectal tumorigenesis may also be driven by the upregulation of the inducible form of cyclooxygenase-2 (COX-2), an enzyme responsible for the conversion of arachidonic acid to PGEs. The present study was undertaken to investigate the expression and activation of the HER family members, and to explore the regulation of COX-2 expression by the HER2 pathway in human colorectal cancer cells. Here, we report that human colorectal cancer cell lines express abundant levels of HER2 and HER3 receptors, and are growth-stimulated by ***recombinant*** neu-differentiation factor-beta 1 (NDF). NDF-treatment of colorectal

TITLE: Complete regression of human B-cell lymphoma xenografts in mice treated with ***recombinant*** anti-CD22 immunotoxin RFB4(dsFv)-PE38 at doses tolerated by cynomolgus monkeys.
AUTHOR: Kreitman R.J.; Wang Q.-C.; FitzGerald D.J.P.; Pastan I.
CORPORATE SOURCE: I. Pastan, Laboratory of Molecular Biology, Division of Basic Sciences, NCI, 37 Convent Drive, Bethesda, MD 20892, United States
SOURCE: International Journal of Cancer, (1999) 81/1 (148-155).
Refs: 34
ISSN: 0020-7136 CODEN: IJCNAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB RFB4(dsFv)-PE38 is a ***recombinant*** immunotoxin in which the variable ***light*** domain (V(L)) is disulfide bonded via cysteine residues to the variable ***heavy*** domain (V(H)), which in turn is fused to PE38, a mutant form of Pseudomonas exotoxin A. RFB4 binds to CD22, which is a differentiation antigen expressed on the majority of B-cell leukemias and lymphomas. To examine the potential efficacy of RFB4(dsFv)-PE38 when administered at a dose schedule appropriate for phase I testing, mice bearing CA46 human CD22+ Burkitt's lymphoma xenografts were treated on alternate days i.v. for 3 doses (QOD x3). Complete regressions were observed in 80% and 100% of mice treated with 200 and 275 .mu.g/kg QOD x3, respectively. The higher dose was 27% of the LD50 and 34% of the LD10 in mice. Because RFB4(dsFv)-PE38 is stable at 37.degree.C, it could also be given by continuous infusion using pumps placed in the peritoneal ***cavity*** ; complete regressions also resulted from this mode of administration. To study toxicology, a pilot toxicology study of RFB4(dsFv)-PE38 was undertaken in cynomolgus monkeys, which like humans but unlike mice have CD22, which binds RFB4. Doses of 100 and 500 .mu.g/kg i.v. QOD x3 were well tolerated, indicating that a dose that cured tumors in mice was tolerated by primates. Based on these preclinical results, RFB4(dsFv)-PE38 is being developed for the treatment of patients with CD22-positive leukemias and lymphomas.

L47 ANSWER 55 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999188700 EMBASE
TITLE: Reconstitution of human pancreatic RNase from two separate fragments fused to different single chain ***antibody*** fragments: On the way to binary immunotoxins.
AUTHOR: Dubel S.
CORPORATE SOURCE: Dr. S. Dubel, Universitat Heidelberg, Molekulare Genetik, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany. s-duebel@sirius.mgen.uni-heidelberg.de
SOURCE: Tumor Targeting, (1999) 4/1 (37-46).
Refs: 46
ISSN: 1351-8488 CODEN: TUTAF
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB By proteolytic cleavage, bovine RNaseA can be dissected into S peptide and S protein, from which the functional RNase can be reconstituted. Here, the cloning of homologous fragments of human placental RNase, huS peptide and huS protein, is described. Further, plasmids based on the pOPE vector family were constructed to express fusion proteins of huS peptide and huS protein with two different scFv (single chain) ***antibody*** fragments. The fusion proteins were produced by secretion to the

periplasmic space of E. coli. Interspecies complementation was demonstrated by combining an scFv fusion protein containing huS peptide or a synthetic huS peptide with bovine S protein. In the reverse setup, RNase activity was reconstituted by combining an scFv fusion protein containing huS protein with synthetic bovine S peptide or synthetic human S peptide. RNase activity was also reconstituted by combining two different scFv antibodies carrying the two fragments of human pancreatic RNase enriched from E. coli periplasmic extracts by immobilised metal affinity chromatography. The results demonstrate the formation of a ***bispecific*** ***antibody*** conjugate with RNase activity. We have already shown that the human pancreatic RNase can efficiently lyse tumour cells in a ***recombinant*** immunotoxin setup. The principle described in this study therefore not only provides a new small linker domain of human origin for the construction of ***bispecific*** antibodies, but might be employed to construct 'binary immunotoxins' with increased affinity, specificity and tumour uptake.

L47 ANSWER 56 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:543225 CAPLUS
DOCUMENT NUMBER: 129:146647
TITLE: Protein fragment complementation assays to detect biomolecular interactions
INVENTOR(S): Michnick, Stephen William Watson; Pelletier, Joelle Nina; Remy, Ingrid
PATENT ASSIGNEE(S): Universite De Montreal, Can.
SOURCE: PCT Int. Appl., 113 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9834120	A1	19980806	WO 1998-CA68	19980202
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2196496	AA	19980731	CA 1997-2196496	19970131
AU 9858505	A1	19980825	AU 1998-58505	19980202
EP 966685	A1	19991229	EP 1998-901905	19980202
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002508832	T2	20020319	JP 1998-532410	19980202

PRIORITY APPLN. INFO.: CA 1997-2196496 A 19970131
WO 1998-CA68 W 19980202

AB We describe a strategy for designing and implementing protein-fragment complementation assays (PCAs) to detect biomol. interactions in vivo and in vitro. The design, implementation and broad applications of this strategy are illustrated with a large no. of enzymes with particular detail provided for the example of murine dihydrofolate reductase (DHFR). Fusion peptides consisting of N- and C-terminal fragments of murine DHFR fused to GCN4 leucine zipper sequences were coexpressed in Escherichia coli grown in minimal medium, where the endogenous DHFR activity was inhibited with trimethoprim. Coexpression of the complementary fusion products restored colony formation. Survival only occurred when both DHFR fragments were present and contained leucine-zipper forming sequences, demonstrating that reconstitution of enzyme activity requires assistance of leucine zipper formation. DHFR fragment-interface point mutants of increasing severity (Ile to Val, ***Ala*** and ***Gly***) resulted in a sequential increase in E. coli doubling times illustrating the successful DHFR fragment reassembly rather than non-specific interactions

between fragments. This assay could be used to study equil. and kinetic aspects of mol. interactions including protein-protein, protein-DNA, protein-RNA, protein-carbohydrate and protein-small mol. interactions, for screening cDNA libraries for binding of a target protein with unknown proteins or libraries of small org. mols. for biol. activity. The selection and design criteria applied here is developed for numerous examples of clonal selection, colorimetric, fluorometric and other assays based on enzymes whose products can be measured. The development of such assay systems is shown to be simple, and provides for a diverse set of protein fragment complementation applications.

L47 ANSWER 57 OF 132 MEDLINE
ACCESSION NUMBER: 1999003244 MEDLINE
DOCUMENT NUMBER: 99003244 PubMed ID: 9786897
TITLE: Mapping the intercellular adhesion molecule-1 and -2 binding ***site*** on the inserted ***domain*** of leukocyte function-associated antigen-1.
AUTHOR: Edwards C P; Fisher K L; Presta L G; Bodary S C
CORPORATE SOURCE: Department of Immunology, Genentech, Inc., South San Francisco, California 94080, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Oct 30) 273 (44) 28937-44.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981201
AB By extensive mutagenic analysis of the inserted ***domain*** (I-***domain***) of the alpha-chain (CD11a) of the leukocyte function-associated antigen-1 (LFA-1), we have defined a putative binding surface for intercellular adhesion molecules 1 and 2 (ICAM-1 and -2). This analysis showed that individually mutating ***Leu*** -205 or Glu-241 to alanine completely abolished LFA-1 binding to ICAM-1 or -2 without affecting I- ***domain*** structure, as assayed by ***antibody*** binding. Mutating Thr-243 to alanine also had a profound effect on LFA-1 binding to ICAM-1 and -2, as seen by complete loss of binding to ICAM-1 and a significant reduction (70% decrease) in binding to ICAM-2. Mutating Glu-146 to alanine reduced LFA-1 binding to ICAM-1 or -2 by 70%, and mutating His-264 or Glu-293 to alanine reduced binding to ICAM-1 or -2 by about 30-40%. Mutating Thr-175 to alanine reduced binding to ICAM-1 by about 30% and binding to ICAM-2 by about 70%. Interestingly, mutating Lys-263 to alanine preferentially abolished LFA-1 binding to ICAM-2. Using these data, we have generated a model of the ***interface*** between the LFA-1 I- ***domain*** and residues in the first ***domain*** of ICAM-1 that have been shown to be critical for this interaction. In addition, this model, together with the ICAM-2 crystal structure, has been used to map residues that are likely to mediate LFA-1 I- ***domain*** binding to ICAM-2.

L47 ANSWER 58 OF 132 MEDLINE
ACCESSION NUMBER: 1999030588 MEDLINE
DOCUMENT NUMBER: 99030588 PubMed ID: 9811819
TITLE: Retinoic acid X receptor in the diploblast, Tripedalia cystophora.
AUTHOR: Kostrouch Z; Kostrouchova M; Love W; Jannini E; Piatigorsky J; Rall J E
CORPORATE SOURCE: Diabetes Branch, National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Nov 10) 95 (23) 13442-7.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States

states.

L47 ANSWER 60 OF 132 MEDLINE
ACCESSION NUMBER: 1998384156 MEDLINE
DOCUMENT NUMBER: 98384156 PubMed ID: 9718313
TITLE: Proteolysis of factor V by cathepsin G and elastase indicates that cleavage at Arg1545 optimizes cofactor function by facilitating factor Xa binding.
AUTHOR: Camire R M; Kalafatis M; Tracy P B
CORPORATE SOURCE: Department of Biochemistry, University of Vermont, College of Medicine, Burlington, Vermont 05405, USA.
CONTRACT NUMBER: R01 HL-52105 (NHLBI)
SOURCE: BIOCHEMISTRY, (1998 Aug 25) 37 (34) 11896-906.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980925
Last Updated on STN: 20000303
Entered Medline: 19980914

AB The single- ***chain*** procofactor factor V is cleaved by thrombin (FVaIIa) at Arg709, Arg1018, and Arg1545 and by a variety of other proteases to generate a cofactor species with various levels of cofactor function. Having demonstrated previously that monocyte-bound forms of cathepsin G and elastase cleave and activate factor V, studies were initiated here using purified proteins to probe factor V structure/function. Electrophoretic, Western blotting, and amino-terminal sequence analyses revealed that cathepsin G cleaves factor V at several sites (Phe1031, Leu1447, Tyr1518, and potentially Tyr696), ultimately generating an amino-terminal 103 kDa ***heavy*** ***chain*** and a carboxy-terminal 80 kDa ***light*** ***chain*** (FVaCG). Elastase also cleaves factor V at several sites (Ile708, Ile819, Ile1484, and potentially Thr678), generating a cofactor species, FVaHNE, with an amino-terminal 102 kDa ***heavy*** ***chain*** and a carboxy-terminal 90 kDa ***light*** ***chain*** . Incubation of FVaIIa with either cathepsin G or elastase resulted in cleavage within the ***heavy*** ***chain*** , releasing peptides of approximately 2000 and approximately 3000 Da, respectively, generating FVaIIa/CG and FVaIIa/HNE. The functional activity of each cofactor species was assessed either by clotting assay or by employing a purified prothrombinase assay using saturating amounts of factor Xa. Significant differences in cofactor function were observed between the two assay systems. Whereas FVaIIa, FVaCG, FVaIIa/CG, FVaHNE, and FVaIIa/HNE all had similar cofactor activities in the purified prothrombinase assay, FVaCG and FVaHNE had no cofactor activity in the clotting-based assay, and FVaIIa/CG and FVaIIa/HNE had approximately 30-35% clotting activity relative to FVaIIa. These disparate results led us to examine the binding interactions of these cofactors with the various prothrombinase components. Kinetic analyses indicated that FVaIIa (Kd(app) = 0.096 nM), FVaIIa/CG (Kd(app) = 0.244 nM), and FVaIIa/HNE (Kd(app) = 0.137 nM) bound to membrane-bound factor Xa much more effectively than FVaCG (Kd(app) = 1.46 nM) and FVaHNE (Kd(app) = 0.818 nM). In contrast, studies of the activated protein C (APC)-catalyzed inactivation of each of the factor V(a) species indicated that they were all equivalent substrates for APC with no differences observed in the rate of inactivation or the cleavage mechanism, suggesting that APC interacts with the ***light*** ***chain*** at a site distinct from factor Xa. The Km values for prothrombin, as well as the kcat values for each of the FV(a) species, were all similar (approximately 0.25 microM and approximately 1900 min-1). In addition, kinetic analyses indicated that whereas FVaCG and FVaHNE exhibited a slightly reduced ability to interact with phospholipid vesicles (approximately 2-3-fold), the remaining FV(a) species ***assembled*** equally well on this surface. Collectively, these data indicate that FVaCG and FVaHNE have a diminished capacity to support factor Xa binding; however, cleavage at Arg1545 and removal of the extended B- ***domain*** in these cofactors

restore near-total factor Xa binding. Thus, cleavage at Arg1545 optimizes cofactor function within prothrombinase by facilitating factor Xa binding to membrane-bound FVa.

L47 ANSWER 61 OF 132 MEDLINE
ACCESSION NUMBER: 1998378534 MEDLINE
DOCUMENT NUMBER: 98378534 PubMed ID: 9710606
TITLE: Identification of an immunoreceptor ***tyrosine*** -based activation motif of K1 transforming protein of Kaposi's sarcoma-associated herpesvirus.
AUTHOR: Lee H; Guo J; Li M; Choi J K; DeMaria M; Rosenzweig M; Jung J U
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772, USA.
CONTRACT NUMBER: CA31363 (NCI)
RR00168 (NCRR)
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1998 Sep) 18 (9) 5219-28.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980917
Last Updated on STN: 19980917
Entered Medline: 19980910
AB Kaposi's sarcoma-associated herpesvirus (KSHV) is consistently identified in Kaposi's sarcoma and body ***cavity*** -based lymphoma. KSHV encodes a transforming protein called K1 which is structurally similar to lymphocyte receptors. We have found that a highly conserved region of the cytoplasmic ***domain*** of K1 resembles the sequence of immunoreceptor ***tyrosine*** -based activation motifs (ITAMs). To demonstrate the signal-transducing activity of K1, we constructed a chimeric protein in which the cytoplasmic tail of the human CD8alpha polypeptide was replaced with that of KSHV K1. Expression of the CD8-K1 chimera in B cells induced cellular ***tyrosine*** phosphorylation and intracellular calcium mobilization upon stimulation with an anti-CD8 ***antibody*** . Mutational analyses showed that the putative ITAM of K1 was required for its signal-transducing activity. Furthermore, ***tyrosine*** residues of the putative ITAM of K1 were phosphorylated upon stimulation, and this allowed subsequent binding of SH2-containing proteins. These results demonstrate that the KSHV transforming protein K1 contains a functional ITAM in its cytoplasmic ***domain*** and that it can transduce signals to induce cellular activation.

L47 ANSWER 62 OF 132 MEDLINE
ACCESSION NUMBER: 1998378516 MEDLINE
DOCUMENT NUMBER: 98378516 PubMed ID: 9710588
TITLE: ErbB-1 and ErbB-2 acquire distinct signaling properties dependent upon their dimerization partner.
AUTHOR: Olayioye M A; Graus-Porta D; Beerli R R; Rohrer J; Gay B; Hynes N E
CORPORATE SOURCE: Friedrich Miescher Institute, CH-4002 Basel, Switzerland.
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1998 Sep) 18 (9) 5042-51.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980917
Last Updated on STN: 20000303
Entered Medline: 19980910
AB The different epidermal growth factor (EGF)-related peptides elicit a diverse array of biological responses as the result of their ability to activate distinct subsets of ErbB receptor dimers, leading to the

recruitment of different intracellular signaling networks. To specifically examine dimerization-dependent modulation of receptor signaling, we constructed NIH 3T3 cell lines expressing ErbB-1 and ErbB-2 singly and in pairwise combinations with each other ErbB family member. This model system allowed the comparison of EGF-activated ErbB-1 with ErbB-1 activated by Neu differentiation factor (NDF)-induced ***heterodimerization*** with ErbB-4. In both cases, ErbB-1 coupled to the adaptor protein Shc, but only when activated by EGF was it able to interact with Grb2. Compared to the rapid internalization of EGF-activated ErbB-1, NDF-activated ErbB-1 showed delayed internalization characteristics. Furthermore, the p85 subunit of phosphatidylinositol kinase (PI3-K) associated with EGF-activated ErbB-1 in a biphasic manner, whereas association with ErbB-1 transactivated by ErbB-4 was monophasic. The signaling properties of ErbB-2 following ***heterodimerization*** with the other ErbB receptors or homodimerization induced by point mutation or monoclonal ***antibody*** treatment were also analyzed. ErbB-2 binding to peptides containing the Src homology 2 domain of Grb2 or p85 and the phosphotyrosine binding domain of Shc varied according to the mode of receptor activation. Finally, tryptic phosphopeptide mapping of both ErbB-1 and ErbB-2 revealed that receptor phosphorylation is dependent on the dimerization partner. Differential receptor phosphorylation may, therefore, be the basis for the differences in the signaling properties observed.

L47 ANSWER 63 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:597024 CAPLUS
DOCUMENT NUMBER: 130:3019
TITLE: Two tyrosines in CD3.epsilon.-ITAM are required to induce T lymphocyte apoptosis
AUTHOR(S): Zheng, Dexian; He, Yiping; Liu, Yanxin; Zheng, Yong; Xiao, Sheng; Liu, Yin; Liu, Shilian
CORPORATE SOURCE: Inst. Basic Med. Sci., Chinese Acad. Med. Sci., Beijing, 100005, Peop. Rep. China
SOURCE: Chinese Science Bulletin (1998), 43(17), 1480-1485
CODEN: CSBUEF; ISSN: 1001-6538
PUBLISHER: Science in China Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB CD3.epsilon. of T cell antigen receptor complex (TCR/CD3) plays an important role in the ***assembling*** of the complex and activation signaling through its conservative immunoreceptor ***tyrosine*** -based activation motif (ITAM) in the cytoplasmic tail. Previous study showed that a chimera mol., consisting of the extracellular-transmembrane ***domain*** of human CD8.alpha. fused to the cytoplasmic ***domain*** of CD3.epsilon., induced apoptosis of T lymphocytes, indicating that apoptotic signals were transduced through the CD3.epsilon.-ITAM. To delineate involvement of the two tyrosines in the apoptotic signaling pathway, cDNAs with mutations at Y170F, Y181F and Y170F/Y181F in CD8.epsilon.-ITAM were made by point mutation and PCR, and then cloned into pcDNA3 eukaryotic expression vectors. Stable expression cell lines were established after transfection of the expression vectors into CD8- Jurkat T lymphocytes. Stimulation of these cell lines with anti-CD8 monoclonal ***antibody*** showed that only the cells with expression of wild type chimera CD8-.epsilon. died by apoptosis, but not those cells with expressions of mutated CD8-.epsilon. chimera, indicating that the two tyrosines in CD3.epsilon.-ITAM were required for the apoptotic signal transduction in T lymphocytes.
REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L47 ANSWER 64 OF 132 MEDLINE
ACCESSION NUMBER: 1998187711 MEDLINE
DOCUMENT NUMBER: 98187711 PubMed ID: 9528862
TITLE: Inhibition of a naturally occurring EGFR oncoprotein by the p185neu ectodomain: implications for subdomain contributions to receptor ***assembly*** .
AUTHOR: O'Rourke D M; Nute E J; Davis J G; Wu C; Lee A; Murali R;

Zhang H T; Qian X; Kao C C; Greene M I
CORPORATE SOURCE: Department of Neurosurgery, University of Pennsylvania, School of Medicine, Philadelphia 19104, USA.
SOURCE: ONCOGENE, (1998 Mar 5) 16 (9) 1197-207.
Journal code: 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980430
Last Updated on STN: 20000303
Entered Medline: 19980420

AB Mutant Epidermal Growth Factor Receptor (EGFR) oncoproteins lacking most of subdomains I and II of the extracellular region, a deletion which includes most of the first of two ***cysteine*** -rich sequences, have been observed in multiple human epithelial tumors, including malignant gliomas. These EGFR oncoproteins, designated deltaEGFR or EGFRvIII, confer increased tumorigenicity in vivo and are often coexpressed with full-length EGFR in human tumors. We have expressed an ectodomain-derived, carboxyl-terminal deletion mutant of the p185neu oncogene (T691stop) in human glioblastoma cells coexpressing endogenous EGFR and activated deltaEGFR oncoproteins. The p185neu ectodomain-derived mutant forms heterodimers with deltaEGFR proteins and reduces the phosphotyrosine content and kinase activity of deltaEGFR monomers. As a consequence of T691stop neu expression and surface localization, cell proliferation in conditions of full growth and reduced serum and anchorage-independent growth in soft agar was reduced in glioblastoma cells expressing either endogenous EGFR alone or coexpressing EGFR and elevated levels of deltaEGFRs. T691stop neu mutant receptors abrogate the dramatic growth advantage conferred by deltaEGFR in vivo, suggesting that physical associations primarily between subdomains III and IV of the p185neu and EGFR ectodomains are sufficient to modulate signaling from activated EGFR complexes. Receptor-based inhibitory strategies exploit the thermodynamic preference for erbB ectodomains to ***heterodimerize*** , thereby creating erbB receptor ***assemblies*** which are defective in signaling and do not internalize. Pharmaceuticals which mimic the p185neu ectodomain may therefore have important therapeutic applications in advanced human malignancies expressing erbB receptors.

L47 ANSWER 65 OF 132 MEDLINE
ACCESSION NUMBER: 1998451282 MEDLINE
DOCUMENT NUMBER: 98451282 PubMed ID: 9780002
TITLE: A novel E2F binding protein with Myc-type HLH motif stimulates E2F-dependent transcription by forming a heterodimer.
AUTHOR: Suzuki M; Okuyama S; Okamoto S; Shirasuna K; Nakajima T; Hachiya T; Nojima H; Sekiya S; Oda K
CORPORATE SOURCE: Department of Biological Science and Technology, Science University of Tokyo, Chiba, Japan.
SOURCE: ONCOGENE, (1998 Aug 20) 17 (7) 853-65.
Journal code: 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981123

AB The human embryonal carcinoma cells NEC14 can be induced to differentiate morphologically by the addition of 10(-2) M N, N'-hexamethylene-bis-acetamide and cease to grow in several days. Transcription factors of the E2F/DP family have been shown to be closely related to the regulation of cell proliferation. To analyse cellular proteins which interact with E2F in NEC14 cells, cDNA clones encoding E2F binding proteins were isolated from a lambdaZAP II NEC14 cell library with the 32P-labeled GST

AUTHOR: ten Dam G B; Poels L G; Wieringa B
CORPORATE SOURCE: Department of Cell Biology and Histology, Faculty of Medical Sciences, University of Nijmegen, The Netherlands.
SOURCE: MOLECULAR BIOLOGY REPORTS, (1998 Nov) 25 (4) 197-204. Journal code: 0403234. ISSN: 0301-4851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 19990712
Entered Medline: 19990621

AB We have designed a new cell surface expression plasmid to study the structural and membrane-topological requirements for functioning of different isoforms of CD45, a leucocyte specific member of the protein ***tyrosine*** phosphatase (PTPase) family of proteins. Use of this vector in cell transfection experiments enabled us to produce multiple CD45 isoforms (ABC, B, Null), with their extracellular segment intact, and the entire membrane spanning and intracellular C-terminal ***domain*** replaced by a GPI-membrane-anchor and VSV-tag. Our strategy facilitated the identification and analysis of chimeric proteins and selection of cell clones from low transfection efficiency experiments. We demonstrate here that simple expression of GPI-anchored CD45 isoforms on transfected Cos-1 cells does not facilitate binding to CD22+ lymphoid cells. This suggests that not only the mere presence of CD45 extracellular domains but also their ***assembly*** into higher order structures at the cell surface, is necessary in order to engage in the recognition and/or signalling processes normally used by B- and T-cells.

L47 ANSWER 70 OF 132 MEDLINE
ACCESSION NUMBER: 1998162553 MEDLINE
DOCUMENT NUMBER: 98162553 PubMed ID: 9501911
TITLE: Crystal structure of the von Willebrand factor A1 domain in complex with the function blocking NMC-4 Fab.
AUTHOR: Celikel R; Varughese K I; Madhusudan; Yoshioka A; Ware J; Ruggeri Z M
CORPORATE SOURCE: Roon Research Center for Arteriosclerosis and Thrombosis, The Scripps Research Institute, La Jolla, California 92037, USA.
SOURCE: NATURE STRUCTURAL BIOLOGY, (1998 Mar) 5 (3) 189-94. Journal code: 9421566. ISSN: 1072-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1OAK
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980410
Last Updated on STN: 19980410
Entered Medline: 19980331

AB The presence of one or more copies of von Willebrand factor type A domains identifies a superfamily of proteins usually involved in biological processes controlled by specific molecular interactions, often adhesive in nature. We have solved the crystal structure of the prototypic von Willebrand factor A1 domain, essential for the antihemorrhagic activity of platelets, in complex with the function blocking ***antibody*** , NMC-4, at 2.2 A resolution. This has led to the recognition of a putative binding groove for the platelet receptor, glycoprotein Ib alpha, formed by two adjacent alpha-helices and a beta-strand. The structure also shows a contact ***interface*** between A1 domain pairs, suggesting a hypothetical mechanism for the regulation of protein assembly and heterologous ligand binding mediated by homophilic interactions of type A domains.

L47 ANSWER 71 OF 132 MEDLINE
ACCESSION NUMBER: 1998172718 MEDLINE

DOCUMENT NUMBER: 98172718 PubMed ID: 9511728
TITLE: Activation of the rat cyclin A promoter by ATF2 and Jun family members and its suppression by ATF4.
AUTHOR: Shimizu M; Nomura Y; Suzuki H; Ichikawa E; Takeuchi A; Suzuki M; Nakamura T; Nakajima T; Oda K
CORPORATE SOURCE: Department of Biological Science and Technology, Science University of Tokyo, Japan.
SOURCE: EXPERIMENTAL CELL RESEARCH, (1998 Feb 25) 239 (1) 93-103. Journal code: 0373226. ISSN: 0014-4827.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980416
Last Updated on STN: 19980416
Entered Medline: 19980408

AB Cyclin A plays an essential role in the G1 to S phase transition in the cell cycle. The expression of cyclin A is restrained during G0 and G1, but steeply induced at the G1/S boundary. Analysis of the rat cyclin A promoter elements with the 5' sequential deletion derivatives of the promoter fused to the luciferase cDNA indicated that the ATF/CRE motif primarily determines the inducibility at G1/S. Gel shift analysis of the complex formed at the ATF/CRE site indicated that the complex was not formed with the G0/G1 cell extract, but maximally formed with the late-G1 cell extract. The complex was supershifted by anti-JunD ***antibody*** , and Western blot analysis of the immune complexes prepared with anti-JunD ***antibody*** revealed the presence of ATF2, suggesting ***heterodimerization*** of JunD with ATF2. The cyclin A promoter in a reporter plasmid was activated by nearly 10-fold in quiescent rat 3Y1 cells by cotransfection with the expression of plasmids encoding ATF2 and Jun family members. In contrast, cotransfection with the ATF4 expression plasmid suppressed the promoter activation mediated by ATF2 and Jun family members. The expression of Jun family members during G1 to S progression was induced biphasically in early and late G1 and the level of JunD increased markedly at the G1/S, while that of ATF family members was gradually increased along with the G1 to S progression. These results indicate that the cyclin A promoter activity is regulated, at least in part, by relative amounts of the ATF and Jun family members.

L47 ANSWER 72 OF 132 MEDLINE
ACCESSION NUMBER: 1998113399 MEDLINE
DOCUMENT NUMBER: 98113399 PubMed ID: 9451439
TITLE: Ligand- and DNA-induced dissociation of RXR tetramers.
AUTHOR: Chen Z P; Iyer J; Bourguet W; Held P; Mioskowski C; Lebeau L; Noy N; Chambon P; Gronemeyer H
CORPORATE SOURCE: Institut de Genetique et de Biology Moleculaire et Cellulaire (IGBMC), CNRS/INSERM/ULP/College de France, Strasbourg.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1998 Jan 9) 275 (1) 55-65. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980210

AB Unliganded bacterially expressed RXR alpha lacking the N-terminal region ***AB*** (apo-RXR alpha delta ***AB***) was found in solution as an apparent mixture of 165 kDa tetramers and 42 kDa monomers which could be quantitatively separated by gel filtration and non-denaturing gel electrophoresis. Under identical conditions both liganded (holo-) and apo-RAR alpha delta ***AB*** were present as single monomeric species. apo-RXR alpha delta ***AB*** tetramers, as well as dimers of the apo-RXR ligand binding domain (apo-LBD), dissociated readily into monomers

when exposed to their cognate ligand 9-cis retinoic acid (9c-RA). The apo-RXR alpha delta ***AB*** tetramer bound only transiently to a cognate DR1 response element, and was converted into DR1-apo-RXR alpha delta ***AB*** homodimer complexes indistinguishable from those generated by cooperative DNA binding of apo-RXR alpha delta ***AB*** monomers. In the absence of DNA, the addition of 9c-RA greatly accelerated the formation of heterodimers with the apo-RAR alpha delta ***AB*** ***heterodimerization*** partner. No RXR alpha delta ***AB*** or RAR alpha delta ***AB*** homodimers could be observed in solution, but upon mixing of the two receptor monomers stable heterodimers could be isolated which bound to DR5 response elements in a highly cooperative manner. In these heterodimers, RXR alpha delta ***AB*** interacted with its cognate ligand as efficiently as in RXR alpha delta ***AB*** homodimers. The presence of ligand did not alter the stability of RXR alpha delta ***AB*** homodimer or RXR alpha delta ***AB*** -RAR alpha delta ***AB*** heterodimer complexes on DR1 and DR5 response elements, respectively. These in vitro data support a model in which RXR tetramers could serve as an inactive pool with the dual function of: (i) rapidly supplying large amounts of RXR ***heterodimerization*** partners upon 9c-RA generation; and (ii) allowing RXR homodimer formation on "accessible" cognate response elements in the absence of 9c-RA. These events may represent a ligand-dependent regulatory mechanism controlling the availability of the promiscuous RXR dimerization partner that is engaged in multiple nuclear receptor signalling pathways.

L47 ANSWER 73 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:729901 CAPLUS
DOCUMENT NUMBER: 128:45063
TITLE: The exon 46-encoded sequence is essential for stability of human erythroid .alpha.-spectrin and heterodimer formation
AUTHOR(S): Wilmotte, Rick; Harper, Sandra L.; Ursitti, Jeanine A.; Marechal, Joelle; Delaunay, Jean; Speicher, David W.
CORPORATE SOURCE: Laboratoire de Genetique Moleculaire Humaine, CNRS URA 1171, Institut Pasteur de Lyon, Lyon, Fr.
SOURCE: Blood (1997), 90(10), 4188-4196
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: Saunders
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human erythroid .alpha.-spectrin alleles responsible for hereditary elliptocytosis (.alpha.HE alleles) undergo increased incorporation into red blood cell membranes when the polymorphism .alpha.LELY (LELY: Low Expression LYon) occurs in trans. The .alpha.LELY polymorphism is characterized by a mutation in exon 40 at codon 1857 (CTA .fwdarw. GTA, ***Leu*** .fwdarw. Val) and the partial (50%) skipping of exon 46, which encodes residues 2177-2182 (Wilmotte et al, J Clin Invest 91:2091, 1993). Both of these peptide sequence alterations are located within the region of the .alpha.-chain involved in initiating heterodimer ***assembly*** , and either or both mutations could potentially contribute to decreased incorporation of .alpha.-chains from the .alpha.LELY allele in heterozygotes into red blood cell membranes. These possibilities were evaluated by testing the protease resistance and in vitro binding properties of normal and mutant ***recombinant*** 4-motif .alpha. subunit peptides contg. the dimer initiation region. The two forms of .alpha. spectrin produced by alternative mRNA splicing of the .alpha.LELY allele were represented by .alpha.18-211857, a peptide with the codon 1857 mutation and retaining the exon 46 encoded sequence, and .alpha.18-211857-.DELTA.46, a peptide carrying both the 1857 codon mutation and the exon 46 deletion. The properties of these two ***recombinant*** peptides were compared with .alpha.18-21, a peptide with the normal sequence at codon 1857 and retaining the exon 46 encoded sequence. The codon 1857 mutation does not adversely affect dimer formation, but it is responsible for the increased trypsin cleavage between the .alpha.IV and .alpha.V domains that was the characteristic feature initially used to identify the .alpha.LELY (Sp.alpha.V/41)

polymorphism (Alloisio et al, J Clin Invest 87:2169, 1991). Deletion of the six amino acids encoded by exon 46 perturbs folding of the .alpha.21 motif, because this region of the .alpha.18-211857-.DELTA.46 peptide is rapidly degraded and this ***recombinant*** peptide is unusually prone to self-aggregation. Exon 46 deletion reduces, but does not eliminate, dimerization. Comparison of mild trypsin proteolytic products from an .alpha.LELY homozygote and the two .alpha.LELY ***recombinant*** peptides strongly suggests that little, if any, of the 50% of the .alpha. chains from the .alpha.LELY allele that contain the exon 46 deletion are incorporated into the mature erythroid membrane. Based on the in vitro anal. of ***recombinant*** .alpha.LELY peptides, the inability of detectable amts. of exon 46- .alpha. chains to ***assemble*** into the mature membrane skeleton in vivo is probably due to a combination of decreased dimer binding affinity and increased proteolytic degrdn. of these mutant chains.

L47 ANSWER 74 OF 132 MEDLINE
ACCESSION NUMBER: 97150826 MEDLINE
DOCUMENT NUMBER: 97150826 PubMed ID: 8995362
TITLE: Mutation of ***tryptophan*** residues in lipoprotein lipase. Effects on stability, immunoreactivity, and catalytic properties.
AUTHOR: Lookene A; Groot N B; Kastelein J J; Olivecrona G; Bruin T
CORPORATE SOURCE: Department of Medical Biochemistry and Biophysics, Umea University, Sweden.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Jan 10) 272 (2) 766-72.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970227
Last Updated on STN: 19980206
Entered Medline: 19970212

AB Previous studies had pointed to an important function of a putative exposed loop in the C-terminal ***domain*** of lipoprotein lipase for activity against emulsified lipid substrates. This loop contains 3 ***tryptophan*** residues (Trp390, Trp393, and Trp394). We have expressed and characterized lipase mutants with ***tryptophan*** to alanine substitutions at positions 55, 114, 382, 390, 393, and 394 and a double mutant at residues 393 and 394. The substitutions in the N-terminal ***domain*** (W55A and W114A) led to poor expression of completely inactive lipase variants. Heparin-Sepharose chromatography showed that mutant W114A eluted at the same salt concentration as inactive wild-type monomers, indicating that this substitution prevented subunit interaction or led to an unstable dimer. In contrast, all mutants in the C-terminal ***domain*** were expressed as mixtures of monomers and dimers similarly to the wild-type. The dimers displayed at least some catalytic activity and had the same apparent heparin affinity as the active wild-type dimers. The mutants W390A, W393A, W394A, and W393A/W394A had decreased reactivity with the monoclonal ***antibody*** 5D2, indicating that the 5D2 epitope is longer than was reported earlier, or that conformational changes affecting the epitope had occurred. The mutants W390A, W393A, W394A, and W393A/W394A had decreased catalytic activity against a synthetic lipid emulsion of long-chain triacylglycerols (IntralipidR) and in particular against rat lymph chylomicrons. The most pronounced decrease of activity was found for the double mutant W393A/W394A which retained only 6% of the activity of the wild-type lipase, while 70% of the activity against water-soluble tributyrylglycerol was retained. In the case of chylomicrons also the affinity for the substrate particles was lowered, as indicated by severalfold higher apparent Km values. This effect was less prominent with the synthetic lipid emulsion. We conclude that the ***tryptophan*** cluster Trp390-Trp393-Trp394 contributes to binding of lipoprotein lipase to lipid/water interfaces. Utilizing different lipid substrates in different physical states, we have demonstrated that the

tryptophan residues in the C-terminal ***domain*** may have a role also in the productive orientation of the enzyme at the lipid/water ***interface*** .

L47 ANSWER 75 OF 132 MEDLINE
ACCESSION NUMBER: 97296332 MEDLINE
DOCUMENT NUMBER: 97296332 PubMed ID: 9151669
TITLE: Transmembrane ***domain*** sequence requirements for activation of the p185c-neu receptor ***tyrosine*** kinase.
AUTHOR: Chen L I; Webster M K; Meyer A N; Donoghue D J
CORPORATE SOURCE: Department of Chemistry and Biochemistry and Center for Molecular Genetics, University of California, San Diego, La Jolla 92093-0367, USA.
CONTRACT NUMBER: CA 40573 (NCI)
SOURCE: JOURNAL OF CELL BIOLOGY, (1997 May 5) 137 (3) 619-31. Journal code: 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970612
Last Updated on STN: 20000303
Entered Medline: 19970602
AB The receptor ***tyrosine*** kinase p185c-neu can be constitutively activated by the transmembrane ***domain*** mutation Val664-->Glu, found in the oncogenic mutant p185neu. This mutation is predicted to allow intermolecular hydrogen bonding and receptor dimerization. Understanding the activation of p185c-neu has assumed greater relevance with the recent observation that achondroplasia, the most common genetic form of human dwarfism, is caused by a similar transmembrane ***domain*** mutation that activates fibroblast growth factor receptor (FGFR) 3. We have isolated novel transforming derivatives of p185c-neu using a large pool of degenerate oligonucleotides encoding variants of the transmembrane ***domain*** . Several of the transforming isolates identified were unusual in that they lacked a Glu at residue 664, and others were unique in that they contained multiple Glu residues within the transmembrane ***domain*** . The Glu residues in the transforming isolates often exhibited a spacing of seven residues or occurred in positions likely to represent the helical ***interface*** . However, the distinction between the sequences of the transforming clones and the nontransforming clones did not suggest clear rules for predicting which specific sequences would result in receptor activation and transformation. To investigate these requirements further, entirely novel transmembrane sequences were constructed based on tandem repeats of simple heptad sequences. Activation was achieved by transmembrane sequences such as [VVVEVVA]n or [VVVEVVV]n, whereas activation was not achieved by a transmembrane ***domain*** consisting only of Val residues. In the context of these transmembrane domains, Glu or Gln were equally activating, while Lys, Ser, and Asp were not. Using transmembrane domains with two Glu residues, the spacing between these was systematically varied from two to eight residues, with only the heptad spacing resulting in receptor activation. These results are discussed in the context of activating mutations in the transmembrane ***domain*** of FGFR3 that are responsible for the human developmental syndromes achondroplasia and acanthosis nigricans with Crouzon Syndrome.

L47 ANSWER 76 OF 132 MEDLINE
ACCESSION NUMBER: 97201485 MEDLINE
DOCUMENT NUMBER: 97201485 PubMed ID: 9049271
TITLE: Characterization of a novel rice bZIP protein which binds to the alpha-globulin promoter.
AUTHOR: Nakase M; Aoki N; Matsuda T; Adachi T
CORPORATE SOURCE: Department of Applied Biological Sciences, School of Agricultural Sciences, Nagoya University, Japan.
SOURCE: PLANT MOLECULAR BIOLOGY, (1997 Feb) 33 (3) 513-22. Journal code: 9106343. ISSN: 0167-4412.

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 20000303
Entered Medline: 19961119

AB Ca2+ binding to the first epidermal growth factor (EGF)-like ***domain*** of factor IX is known to be required for biological activity, but the mechanism by which Ca2+ contributes to factor IX function has remained unclear. We have studied recombinant factor IX mutants which lack Ca2+ binding to the first EGF-like ***domain*** , due to a replacement of Asp64 by Glu, Lys, or Val. The purified mutants (factors IX D64E, D64K, and D64V), were compared to plasma-derived and recombinant wild-type factor IX with regard to a number of metal-ion dependent functional parameters. In the presence of Mg2+, the activated mutants were indistinguishable from normal factor IXa in hydrolyzing the synthetic substrate CH3-SO2- ***Leu*** -Gly- ***Arg*** -p-nitroanilide. Replacing Mg2+ by Ca2+ further stimulated the activity of normal factor IXa but not of mutant factor IXa. In factor VIII-independent factor X activation, factor IXa D64K and D64E displayed reduced catalytic activity compared to normal factor IXa (apparent kcat/Km approximately 1, 2, and 4 x 10(3) M-1 s-1, respectively). In the presence of factor VIIIa, factor X activation rates by normal and mutant factor IXa were stimulated by factor VIIIa to a different extent (approximately700- and 200-fold, respectively), indicating that Asp64 replacements affect the interaction with factor VIIIa. This possibility was addressed in inhibition studies employing synthetic peptides comprising the factor IXa-binding motifs of factor VIII ***heavy*** or ***light*** chains. Whereas the ***heavy*** ***chain*** peptide (Ser558-Gln565) inhibited factor VIII-dependent factor X activation by normal and mutant factor IXa with similar efficiency, the ***light*** ***chain*** peptide (Lys1804-Lys1818) inhibited normal factor IXa 2-3-fold more efficiently than did mutant factor IXa. This indicates that the reduced response to factor VIIIa may be due to impaired binding of mutant factor IXa to the factor VIII ***light*** ***chain*** . This was further explored in direct binding studies. In the presence of Mg2+, normal and mutant factor IXa were similar in binding to the factor VIII ***light*** ***chain*** . However, in the presence of Ca2+, factor IXa mutants were less efficient than normal factor IXa, which was illustrated by a 4-5-fold lower affinity than normal factor IXa for factor VIII ***light*** ***chain*** . Collectively, our data demonstrate that a number of factor IXa functions, including enzymatic activity and ***assembly*** into the factor IXa-factor VIIIa complex, are dependent on Ca2+ binding to the first EGF-like ***domain*** of factor IX.

L47 ANSWER 85 OF 132 MEDLINE
ACCESSION NUMBER: 96312880 MEDLINE
DOCUMENT NUMBER: 96312880 PubMed ID: 8703938
TITLE: A mutational analysis of the binding of two different proteins to the same ***antibody*** .
AUTHOR: Dall'Acqua W; Goldman E R; Eisenstein E; Mariuzza R A
CORPORATE SOURCE: Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville 20850, USA.
CONTRACT NUMBER: GM52801 (NIGMS)
RR08937 (NCRR)
SOURCE: BIOCHEMISTRY, (1996 Jul 30) 35 (30) 9667-76.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960919
Last Updated on STN: 19960919
Entered Medline: 19960910

AB The crystal structures of the complexes between the anti-hen egg white lysozyme (HEL) ***antibody*** D1.3 and HEL and between D1.3 and the

anti-D1.3 ***antibody*** E5.2 have shown that D1.3 contacts these two proteins through essentially the same set of combining site residues [Fields, B. A., Goldbaum, F. A., Ysern, X., Poljak, R. J., & Mariuzza, R. A. (1995) Nature 374, 739-742]. To probe the relative contribution of individual residues to complex stabilization, single alanine substitutions were introduced in the combining site of D1.3, and their effects on affinity for HEL and for E5.2 were measured using surface plasmon resonance detection, fluorescence quench titration, or sedimentation equilibrium. The energetics of the binding to HEL are dominated by only 3 of the 13 contact residues tested (delta Gmutant-delta Gwild type > 2.5 kcal/mol): VLW92, VHD100, and VHY101. These form a patch at the center of the ***interface*** and are surrounded by residues whose apparent contributions are much less pronounced (< 1.5 kcal/mol). This contrasts with the interaction of D1.3 with E5.2 in which most the contact residues (11 of 15) were found to play a significant role in ligand binding (> 1.5 kcal/mol). Furthermore, even though D1.3 contacts HEL and E5.2 in very similar ways, the functionally important residues of D1.3 are different for the two interactions, with only substitutions at D1.3 positions VH100 and VH101 greatly affecting binding to both ligands. Thus, the same protein may recognize different ligands in ways that are structurally similar yet energetically distinct.

L47 ANSWER 86 OF 132 MEDLINE
ACCESSION NUMBER: 96224130 MEDLINE
DOCUMENT NUMBER: 96224130 PubMed ID: 8621555
TITLE: The Goodpasture autoantigen. Structural delineation of two immunologically privileged epitopes on alpha3(IV) ***chain*** of type IV collagen.
AUTHOR: Kalluri R; Sun M J; Hudson B G; Neilson E G
CORPORATE SOURCE: Penn Center for Molecular Studies of Kidney Diseases, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.
CONTRACT NUMBER: DK-07006 (NIDDK)
DK-18381 (NIDDK)
DK-30280 (NIDDK)
+
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Apr 12) 271 (15) 9062-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960627
Last Updated on STN: 19980206
Entered Medline: 19960620

AB The family of type IV collagen comprises six chains numbered alpha1 through alpha6. The alpha3(IV) NC1 ***domain*** is the primary target antigen for autoantibodies from patients with anti-basement membrane disease and Goodpasture syndrome. Earlier peptide studies suggested that the last 36 amino acids of the alpha3 NC1 ***domain*** probably contains one recognition site for Goodpasture autoantibodies, and an algorithm analysis of secondary structure from a later study predicted a second possible upstream epitope near the triple helix junction. We have used several analytic approaches to evaluate the likelihood of two immunologic epitopes for the Goodpasture antigen. In our first set of studies, peptide antibodies directed against these two putative regions co-inhibited Goodpasture autoantibodies binding to denatured human alpha3(IV) NC1 monomer by nearly 80%, with the helix-junction region of the alpha3 NC1 ***domain*** contributing 26% of the binding sites and the C-terminal region contributing the remaining 50%. Second, both of these candidate regions are normally sequestered within the associated alpha3(IV) NC1 hexamer but become more visible for binding by anti-peptide antibodies upon their dissociation, a property that is shared by the Goodpasture autoantibodies. Third, segment deletions of recombinant alpha3 NC1 ***domain*** further confirmed the presence of two serologic

TITLE: The two membrane isoforms of human IgE ***assemble*** into functionally distinct B cell antigen receptors.
AUTHOR: Batista F.D.; Anand S.; Presani G.; Efremov D.G.; Burrone O.R.
CORPORATE SOURCE: Dr. O.R. Burrone, ICGEB, Area Science Park, Padriciano 99, 34012 Trieste, Italy
SOURCE: Journal of Experimental Medicine, (1996) 184/6 (2197-2205).
Refs: 42
ISSN: 0022-1007 CODEN: JEMEAV
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The human C.epsilon. gene expresses two membrane IgE heavy ***chain*** mRNAs which differ in the sequence that encodes their extracellular membrane-proximal ***domain*** . In the long IgE isoform (m(L)IgE), this ***domain*** contains a stretch of 52 amino acids which are absent in the short variant (m(S)IgE). We have now generated B cell transfectoma cell lines that express these two isoforms and show that both types of mIgE form functional B cell antigen receptors (BCR). Both receptors associate with the Ig-.alpha./Ig-.beta. heterodimer, as well as with protein kinases that are capable of phosphorylating this complex. Upon their cross-linking, both receptors can activate protein ***tyrosine*** kinases that phosphorylate the same substrate proteins. Both IgE receptors also associate with two novel proteins that do not bind to mIgM. Apart from these similarities, the two IgE-BCRs show several differences of which some are analogous to the differences between the IgM- and IgD-BCRs. First, the m(S)IgE is transported to the cell surface at a higher rate than the m(L)IgE. Second, the two IgE-BCRs associate with differently glycosylated Ig-.alpha. proteins, the m(L)IgE associates with the completely glycosylated form, whereas the m(S)IgE associates with an Ig-.alpha. glycoform that is partially sensitive to endoglycosidase H. Third, the kinetics of protein ***tyrosine*** phosphorylation induced by receptor cross-linking is significantly different for the two IgE-BCRs. Finally, cross-linking of the m(S)IgE-BCR leads to growth inhibition of the B cell transfectoma, whereas signaling through the m(L)IgE-BCR does not affect the cellular proliferation. These data show that the two human membrane IgE isoforms ***assemble*** into functionally distinct antigen receptors which can induce different cellular responses.

L47 ANSWER 89 OF 132 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:118311 BIOSIS
DOCUMENT NUMBER: PREV199698690446
TITLE: The sequence Glu-1811-Lys-1818 of human blood coagulation factor VIII comprises a binding site for activated factor IX.
AUTHOR(S): Lenting, Peter J.; Van De Loo, Jan-Willem H. P.; Donath, Marie-Jose S. H.; Van Mourik, Jan A.; Mertens, Koen (1)
CORPORATE SOURCE: (1) Dep. Blood Coagulation, Central Lab. Netherlands Red Cross Blood Transfusion Serv., Plesmanlaan 125, 1066 CX Amsterdam Netherlands
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 4, pp. 1935-1940.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB In previous studies we have shown that the interaction between factors IXa and VIII involves the light ***chain*** of factor VIII and that this interaction is inhibited by the monoclonal ***antibody*** CLB-CAg A against the factor VIII region Gln-1778-Asp-1840 (Lenting, P. J., Donath, M. J. S. H., van Mourik, J. A., and Mertens, K. (1994) J. Biol. Chem. 269, 7150-7155). Employing distinct recombinant factor VIII fragments, we now have localized the epitope of this ***antibody*** more precisely between the A3 ***domain*** residues Glu-1801 and Met-1823. Hydropathy analysis indicated that this region is part of a major hydrophilic exosite within the A3 ***domain*** . The interaction of factor IXa with this

exosite was studied by employing overlapping synthetic peptides encompassing the factor VIII region ***Tyr*** -1786-Ala-1834. Factor IXa binding was found to be particularly efficient to peptides corresponding to the factor VIII sequences Lys-1804-Lys-1818 and Glu-1811-Gln-1820. The same peptides proved effective in binding ***antibody*** CLB-CAg A. Further analysis revealed that peptides Lys-1804-Lys-1818 and Glu-1811-Gln-1820 interfere with binding of factor IXa to immobilized factor VIII light ***chain*** (K-i apprxeq 0.2 mM and 0.3 mM, respectively). Moreover, these peptides inhibit factor X activation by factor IXa in the presence of factor VIIIa (K-i apprxeq 0.2 mM and 0.3 mM, respectively) but not in its absence. Equilibrium binding studies revealed that these two peptides bind to the factor IX zymogen and its activated form, factor IXa, with the same affinity (apparent K-d apprxeq 0.2 mM), whereas the complete factor VIII light ***chain*** displays preferential binding to factor IXa. In conclusion, our results demonstrate that peptides consisting of the factor VIII light ***chain*** residues Lys-1804-Lys-1818 and Glu-1811Gln-1820 share a factor IXa binding site that is essential for the ***assembly*** of the factor X-activating factor IXa-factor VIIIa complex. We propose that the overlapping sequence Glu-1811-Lys-1818 comprises the minimal requirements for binding to activated factor IX.

L47 ANSWER 90 OF 132 MEDLINE
ACCESSION NUMBER: 96266493 MEDLINE
DOCUMENT NUMBER: 96266493 PubMed ID: 8683598
TITLE: Rearrangement of the former VL ***interface*** in the solution structure of a camelised, single ***antibody*** VH domain.
AUTHOR: Riechmann L
CORPORATE SOURCE: MRC Laboratory of Molecular Biology, Cambridge, UK.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1996 Jun 28) 259 (5) 957-69.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960828
Last Updated on STN: 19960828
Entered Medline: 19960820

AB The solution structure of the isolated ***antibody*** ***heavy*** chain variable domain (VH)-P8 was determined by NMR spectroscopy. The VH had previously been modified (camelised) at three positions in its former ***antibody*** ***light*** chain variable domain (VL) ***interface*** to reduce hydrophobicity by mimicking camelid ***heavy*** chains naturally devoid of ***light*** chains. The architecture of two pleated beta-sheets and the conformation of the H1 and H2 loops in VH-P8 are very similar to those in non-camelised, VL-associated VH domains. Major differences concern the H3 loop, which no longer points towards the now absent VL, and three residues in the former VL ***interface*** . The side-chains of Val37 and Trp103 are buried and the Arg38 side-chain exposed in VH-P8. In non-camelised, VL-associated VH domains the side-chains of Val37 and Trp103 are in contact with the VL while the Arg38 side-chain is buried within the VH. Reorientation of Trp103 is due to the local structure in the beta-bulge of strand G. Reorientation of Val37 and Arg38 is caused by a disruption of regular beta-structure in strand C opposite the beta-bulge in strand C'. These changes, combined with the more hydrophilic side-chains of the camelised residues, reduce hydrophobicity and prevent non-specific binding of camelised VH domains, which proved critical for their use as small recognition units. The VH-P8 structure also indicates structural reasons for two other mutations specific for ***light*** -chain-lacking camel immunoglobins. Absence of the VH-typical Arg94/Asp101 salt bridge at the base of the H3 loop in VH-P8 may explain why a positively charged residue at position 94 is not conserved in camels. Reorientation of Val37 suggests a function of the camel-specific phenylalanine residue at this position in the hydrophobic core of ***light*** -chain-lacking camel ***heavy***

chains.

L47 ANSWER 91 OF 132 MEDLINE
ACCESSION NUMBER: 96184158 MEDLINE
DOCUMENT NUMBER: 96184158 PubMed ID: 8621240
TITLE: A bivalent single-chain antibody-toxin specific for ErbB-2 and the EGF receptor.
AUTHOR: Schmidt M; Hynes N E; Groner B; Wels W
CORPORATE SOURCE: Institute for Experimental Cancer Research, Tumor Biology Center, Freiburg, Germany.
SOURCE: INTERNATIONAL JOURNAL OF CANCER, (1996 Feb 8) 65 (4) 538-46.
Journal code: 0042124. ISSN: 0020-7136.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960627
Last Updated on STN: 20020420
Entered Medline: 19960614
AB ErbB-2 and EGF receptors are often co-expressed in human tumors and have been shown to synergize in the transformation of cells in experimental model systems. Transactivation of ErbB-2 can occur via ligand-induced ***heterodimerization*** with EGF receptor or other members of the ErbB family of receptor ***tyrosine*** kinases. We have previously described the potent anti-tumoral activity of the monospecific single-chain antibody-toxins scFv(FRP5)-ETA and scFv(225)-ETA binding to, respectively, ErbB-2 and the EGF receptor. Here we report the construction and functional characterization of a novel bivalent, ***bispecific*** single-chain antibody-toxin, scFv2(FRP5/225)-ETA. The fusion protein consists of 2 scFv domains specific for ErbB-2 and the EGF receptor linked to a modified Pseudomonas exotoxin A. ScFv2(FRP5/225)-ETA displayed in vitro cell killing activity on tumor cells overexpressing either ErbB-2 or the EGF receptor similar to that of the monospecific toxins. It was more potent in vitro and in vivo in inhibiting the growth of tumor cells expressing both receptors. Treatment of A431 cells with scFv2(FRP5/225)-ETA led to an increase in EGF receptor and ErbB-2 phosphotyrosine content, most likely via the induction of receptor heterodimers. This may explain the enhanced toxicity of the ***bispecific*** antibody-toxin.

L47 ANSWER 92 OF 132 MEDLINE
ACCESSION NUMBER: 96201132 MEDLINE
DOCUMENT NUMBER: 96201132 PubMed ID: 8619629
TITLE: Long loop residues 33-58 in the human glycoprotein hormone common alpha subunit contain structural components for subunit ***heterodimerization*** and human follitropin-receptor binding.
AUTHOR: Liu C; Dias J A
CORPORATE SOURCE: Department of Biomedical Sciences, State University of New York at Albany 12201-0509, USA.
CONTRACT NUMBER: HD-18407 (NICHD)
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1996 May 1) 329 (1) 127-35.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960620
Last Updated on STN: 19960620
Entered Medline: 19960612
AB The family of human glycoprotein hormones that includes follitropin (FSH) are heterodimeric proteins, each composed of single alpha and beta subunits that are non-covalently linked but tightly associated. Previous

studies by this laboratory, which used a synthetic peptide approach, suggested that residues 51-58 of the long loop of FSH alpha (aa 33-58) were the minimal alpha-subunit contact area between the subunits. Since carbohydrate at N52 is important for receptor activation but not for receptor binding, a link between receptor activation and heterodimer ***assembly*** was established. To address this issue, four composite ***alanine*** substitution mutants, 37YPTPL41/37APAPA41, 46TML48/46AAA48, 49VQK51/49AAA51, and 55SES57/55AAA57, were constructed by site-specific mutagenesis and expressed in insect cells. With the exception of the TML mutant, all alpha-subunit forms were produced at a level similar to that of the wild-type alpha subunit (10 micromilligram(s)). The TML mutant was not secreted. When coexpressed with the human FSH (hFSH) beta subunit the 49VQK51/49AAA51 mutant and wild-type hFSH were expressed at similar levels (1-3 micromilligram(s)). In contrast, the 55SES57/65AAA57 mutation evidenced barely detectable levels of heterodimeric hFSH, and 37YPTPL41/37APAPA41 was not detectable as heterodimer, measured in a capture enzyme-linked immunosorbent assay format that detects only heterodimeric hormone. The 49VQK51/49AAA51 mutant was devoid of receptor-binding activity, suggesting that these residues are a key alpha-subunit determinant for follitropin-receptor interaction. The 55SES57/55AAA57 mutant, though scarcely made, retained receptor-binding activity comparable to the wild-type hormone. This work demonstrates for the first time a receptor-binding region in the FSH alpha subunit, within sequence 49VQK51. Residues within 55SES57 and 37YPTPL41 are involved in subunit ***assembly*** . Homology modeling of FSH, based on the human chorionic gonadotropin crystal structure, revealed that the FSH receptor-binding site is composed of residues from both subunits ***assembled*** through subunit association.

L47 ANSWER 93 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96270040 EMBASE
DOCUMENT NUMBER: 1996270040
TITLE: Coadministration of interleukin-6 (IL-6) and soluble IL-6 receptor delays progression of wobbler mouse motor neuron disease.
AUTHOR: Ikeda K.; Kinoshita M.; Tagaya N.; Shiojima T.; Taga T.; Yasukawa K.; Suzuki H.; Okano A.
CORPORATE SOURCE: Fourth Dept. of Internal Medicine, Toho University Ohashi Hospital, 2-17-6, Ohashi,Meguro-ku, Tokyo 153, Japan
SOURCE: Brain Research, (1996) 726/1-2 (91-97).
ISSN: 0006-8993 CODEN: BRREAP
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 008 Neurology and Neurosurgery
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Interleukin-6 (IL-6), a multipotential cytokine, initiates signal transduction pathways similar to those of ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF). These molecules share the signal transducing receptor component, gp130, IL-6 triggers homodimerization of gp130, whereas CNTF and LIF induce ***heterodimerization*** of gp130 and LIF receptor. Although CNTF or LIF treatment attenuates motor deficits in wobbler mouse motor neuron disease (MND), neuroprotective effects of IL-6 on this animal have nol yet been clarified. Here we studied whether simultaneous treatment with IL-6 and soluble IL-6 receptor (sIL-6R) can ameliorate symptomatic and neuropathological changes in wobbler mouse MND. After clinical diagnosis at postnatal age 3-4 weeks, wobbler mice received subcutaneous injection with human ***recombinant*** IL-6 (1.0 mg/kg), human sIL- 6R (0.5 mg/kg), IL-6 + sIL-6R or vehicle, daily for 4 weeks in a blind fashion. Compared to vehicle, coadministration with IL-6 and sIL-6R potentiated grip strength, attenuated muscle contractures in the forelimbs, reduced denervation muscle atrophy and prevented degeneration of spinal motor neurons. Single administration with IL-6 or sIL-6R did not retard the symptomatic and neuropathological progression, although IL-6 treated mice

did not raise anti-IL-6 antibodies. Treatment with IL-6 + sIL-6R, but not with IL-6 or sIL-6R alone delayed progression of wobbler mouse MND. Our results indicate that the neuroprotective mechanism for IL-6/sIL-6R on wobbler mouse MND differs from that of CNTF or LIF alone. We hypothesize that IL-6/sIL-6R complex may function on motor neurons through activation and homodimerization of gp130.

L47 ANSWER 94 OF 132 MEDLINE
ACCESSION NUMBER: 96301834 MEDLINE
DOCUMENT NUMBER: 96301834 PubMed ID: 8723318
TITLE: Comparative interaction kinetics of two ***recombinant*** Fabs and of the corresponding antibodies directed to the coat protein of tobacco mosaic virus.
AUTHOR: Chatellier J; Rauffer-Bruyere N; Van Regenmortel M H; Altschuh D; Weiss E
CORPORATE SOURCE: Institut de Biologie Moleculaire et Cellulaire du CNRS, Laboratoire d'Immunochimie, Strasbourg, France.
SOURCE: JOURNAL OF MOLECULAR RECOGNITION, (1996 Jan-Feb) 9 (1) 39-51.
Journal code: 9004580. ISSN: 0952-3499.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19990129
Entered Medline: 19961203
AB Two ***recombinant*** Fab fragments, 57P and 174P, recognizing peptide 134-146 of the coat protein of tobacco mosaic virus have been cloned, sequenced and expressed in Escherichia coli. They differ by 15 amino acid changes in the sequence of their variable region. The interaction kinetics of the Fabs with the wild-type and four mutant peptides have been compared using a BIAcoreTM biosensor instrument. The ***recombinant*** Fab 174P had the same reactivity as the Fab fragment obtained by enzymatic cleavage of monoclonal ***antibody*** 174P. The two ***recombinant*** Fabs recognized the various peptides in the same ranking order but Fab 174P consistently dissociated somewhat faster from the peptides compared to Fab 57P. The two whole antibodies showed the same relative differences in reactivity as the two ***recombinant*** Fabs. The location of amino acid changes was visualized on a model structure of the Fab. Differences in dissociation rates of the two antibodies are most likely due to changes located at the periphery of the antigen-combining site and/or at the ***interface*** between the ***light*** and ***heavy*** chain domains. Our results demonstrate the feasibility of detecting very small differences in binding affinity by the biosensor technology, which is a prerequisite for assessing the functional effect of limited structural changes.

L47 ANSWER 95 OF 132 MEDLINE
ACCESSION NUMBER: 96163541 MEDLINE
DOCUMENT NUMBER: 96163541 PubMed ID: 8576577
TITLE: Structural analysis of gamma delta TCR using a novel set of TCR gamma and delta chain-specific monoclonal antibodies generated against soluble gamma delta TCR. Evidence for a specific conformation adopted by the J delta 2 region and for a V delta 1 polymorphism.
AUTHOR: Romagne F; Peyrat M A; Leget C; Davodeau F; Houde I; Necker A; Hallet M M; Vie H; Bonneville M
CORPORATE SOURCE: Immunotech SA, Marseilles, France.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1996 Jan 16) 189 (1) 25-36.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960321
Last Updated on STN: 19960321
Entered Medline: 19960313

AB We recently showed that secretion of non-chimeric disulfide-linked human gamma delta TCR ('soluble' TCR, sTCR) comprising V gamma 9 and V delta 2 regions could be achieved by simply introducing translational termination codons upstream from the sequences encoding TCR transmembrane region. Here we extended these findings by demonstrating efficient secretion and ***heterodimerization*** of gamma delta sTCR comprising V gamma 8, V delta 1 and V delta 3 regions, obtained via the same strategy. After immunization against immunoaffinity-purified soluble TCR, several hundreds of TCR-specific monoclonal antibodies (***mAb***) were generated, which fell in at least seven groups. One set of ***mAb*** was directed against a V gamma 8-specific epitope. Strikingly, despite the high degree of sequence homology between V gamma 8 and other V gamma I domains, none of these ***mAb*** were crossreactive with other members of the V gamma I family. Three other sets of mAbs were shown to recognize delta chains comprising V delta 1, V delta 2 and V delta 3 regions respectively, regardless of their junctional sequence or of the gamma chain to which they were paired. Among the V delta 1-specific ***mAb*** , some specifically recognized V delta 1D delta J delta C delta chains while others reacted with both V delta 1 D delta J delta C delta and V delta 1J alpha C alpha chains, which suggested V domain conformational alterations induced by the C region. Moreover, reactivity of one V delta 1-specific ***mAb*** (#R6.11) was affected by a polymorphic residue located on the predicted CDR4 loop of the V delta region. Two delta chain-specific ***mAb*** (#178 and #515) showed a highly unusual reactivity, which was negatively affected by particular V delta and J delta sequences: (i) ***mAb*** #515 and #178 recognized all TCR delta chains except those comprising V delta 1 or V delta 2 regions, respectively and (ii) within TCR delta chains carrying 'permissive' V delta regions, none of those comprising the J delta 2 region were recognized by #515 and/or #178 mAbs, which suggested a highly specific conformation adopted by this particular J delta sequence. Apart from its usefulness in TCR structural studies, this novel set of ***mAb*** represents an important tool for the characterization and isolation of gamma delta T cells expressing particular combinations of V gamma/V delta regions and for analysis of V alpha/V delta usage by alpha beta T cells. Moreover, since our present data strongly suggest that gamma delta TCR are easier to obtain in a soluble form than alpha beta TCR, an efficient strategy for the generation of V alpha region-specific ***mAb*** might be to immunize with chimeric gamma delta sTCR comprising particular V alpha regions.

L47 ANSWER 96 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95186955 EMBASE
DOCUMENT NUMBER: 1995186955
TITLE: A cytoplasmic region of the Na,K-ATPase .alpha.-subunit is necessary for specific .alpha./.alpha. association.
AUTHOR: Koster J.C.; Blanco G.; Mercer R.W.
CORPORATE SOURCE: Dept. of Cell Biology and Physiology, Washington Univ. School of Medicine, Box 8228, 660 S. Euclid Avenue,St. Louis, MO 63110, United States
SOURCE: Journal of Biological Chemistry, (1995) 270/24 (14332-14339).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB While most structural studies of the Na,K-ATPase support a subunit stoichiometry of one .alpha.-subunit to one .beta.-subunit, the exact quaternary structure of the Na,K-ATPase and its relevance to enzyme function is the subject of much debate. Formation of a higher order enzyme complex is supported by our previous study demonstrating specific .alpha./.alpha. interactions among the rat Na,K-ATPase isoforms (.alpha.1,

.alpha.2, .alpha.3), expressed in virally infected Sf-9 insect cells and among native .alpha. isoforms in rat brain (1). This detergent-resistant association was not observed in insect cells coexpressing the homologous gastric H,K-ATPase .alpha.-subunit, nor was it dependent on the coexpression of the .beta.-subunit. To delineate domains necessary for .alpha./.alpha. ***assembly*** , a series of H,K-ATPase. Na,K-ATPase chimeras were constructed by combining the N-terminal, cytoplasmic midregion and C- terminal segments derived from the Na,K-ATPase (N) and the H,K-ATPase (H) .alpha.- polypeptides (HNN, HNH, NHH, NHN, and HHN). The .alpha.-subunit chimeras were coexpressed with the Na,K-ATPase .alpha.1-subunit in Sf-9 cells using the baculovirus expression system. Specific and detergent-stable association is observed between the Na,K-ATPase .alpha.-subunit and the HNN and HNH chimeras, but not with the NHH, NHN, or HHN chimeras. Consistent with the Na,K-ATPase cytoplasmic ***domain*** as being necessary for .alpha./.alpha. interactions, the full- length .alpha.-subunit stably associates with an .alpha. N-terminal deletion mutant (.DELTA.Gly2-Leu273), but not with an .alpha. cytoplasmic deletion mutant (.DELTA.Arg350-Pro785). In addition, the naturally occurring C-terminal truncated .alpha.1 isoform, .alpha.1T (.DELTA.Gly554 to C terminus), does not associate with the .alpha.1-subunit in Sf-9 cells coexpressing both polypeptides. Thus, a cytoplasmic region in the .alpha.-subunit (Gly554-Pro785) is necessary for specific .alpha./.alpha. association. The same cytoplasmic region contains a strongly hydrophobic segment that, by analogy with oligomerization of water-soluble proteins, may form the interface of the extramembranous .alpha./.alpha. contact site.

L47 ANSWER 97 OF 132 MEDLINE
ACCESSION NUMBER: 96003862 MEDLINE
DOCUMENT NUMBER: 96003862 PubMed ID: 7568216
TITLE: Specificity of dimer formation in tropomyosins: influence of alternatively spliced exons on homodimer and heterodimer assembly.
AUTHOR: Gimona M; Watakabe A; Helfman D M
CORPORATE SOURCE: Cold Spring Harbor Laboratory, NY 11724, NY, USA.
CONTRACT NUMBER: CA58607 (NCI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1995 Oct 10) 92 (21) 9776-80. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 19951227
Entered Medline: 19951114
AB Tropomyosins consist of nearly 100% alpha-helix and assemble into parallel and in-register coiled-coil dimers. In vitro it has been established that nonmuscle as well as native muscle tropomyosins can form homodimers. However, a mixture of muscle alpha and beta tropomyosin subunits results in the formation of the thermodynamically more stable alpha/beta heterodimer. Although the assembly preference of the muscle tropomyosin heterodimer can be understood thermodynamically, the presence of multiple tropomyosin isoforms expressed in nonmuscle cells points toward a more complex principle for determining dimer formation. We have investigated the dimerization of rat tropomyosins in living cells by the use of epitope tagging with a 16-aa sequence of the influenza hemagglutinin. Employing transfection and immunoprecipitation techniques, we have analyzed the dimers formed by muscle and nonmuscle tropomyosins in rat fibroblasts. We demonstrate that the information for homo- versus ***heterodimerization*** is contained within the tropomyosin molecule itself and that the information for the selectivity is conferred by the alternatively spliced exons. These results have important implications for models of the regulation of cytoskeletal dynamics.

L47 ANSWER 98 OF 132 MEDLINE

ACCESSION NUMBER: 95166250 MEDLINE
DOCUMENT NUMBER: 95166250 PubMed ID: 7862156
TITLE: Subcellular localization of the alpha and beta subunits of the acute myeloid leukemia-linked transcription factor PEBP2/CBF.
AUTHOR: Lu J; Maruyama M; Satake M; Bae S C; Ogawa E; Kagoshima H; Shigesada K; Ito Y
CORPORATE SOURCE: Department of Viral Oncology, Kyoto University, Japan.
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1995 Mar) 15 (3) 1651-61.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950404
Last Updated on STN: 19950404
Entered Medline: 19950323

AB Each of the two human genes encoding the alpha and beta subunits of a heterodimeric transcription factor, PEBP2, has been found at the breakpoints of two characteristic chromosome translocations associated with acute myeloid leukemia, suggesting that they are candidate proto-oncogenes. Polyclonal antibodies against the alpha and beta subunits of PEBP2 were raised in rabbits and hamsters. Immunofluorescence labeling of NIH 3T3 cells transfected with PEBP2 alpha and -beta cDNAs revealed that the full-size alpha A1 and alpha B1 proteins, the products of two related but distinct genes, are located in the nucleus, while the beta subunit is localized to the cytoplasm. Deletion analysis demonstrated that there are two regions in alpha A1 responsible for nuclear accumulation of the protein: one mapped in the region between amino acids 221 and 513, and the other mapped in the Runt domain (amino acids 94 to 221) harboring the DNA-binding and the ***heterodimerizing*** activities. When the full-size alpha A1 and beta proteins are coexpressed in a single cell, the former is present in the nucleus and the latter still remains in the cytoplasm. However, the N- or C-terminally truncated alpha A1 proteins devoid of the region upstream or downstream of the Runt domain colocalized with the beta protein in the nucleus. In these cases, the beta protein appeared to be translocated into the nucleus passively by binding to alpha A1. The chimeric protein containing the beta protein at the N-terminal region generated as a result of the inversion of chromosome 16 colocalized with alpha A1 to the nucleus more readily than the normal beta protein. The implications of these results in relation to leukemogenesis are discussed.

L47 ANSWER 99 OF 132 MEDLINE
ACCESSION NUMBER: 95352511 MEDLINE
DOCUMENT NUMBER: 95352511 PubMed ID: 7626514
TITLE: Receptor mediated genomic action of the 1,25(OH)2D3 hormone: expression of the human vitamin D receptor in E. coli.
AUTHOR: Hsieh J C; Nakajima S; Galligan M A; Jurutka P W; Haussler C A; Whitfield G K; Haussler M R
CORPORATE SOURCE: Department of Biochemistry, College of Medicine, University of Arizona, Tucson 85724, USA.
CONTRACT NUMBER: AR15781 (NIAMS)
DK33351 (NIDDK)
DK40372 (NIDDK)
SOURCE: JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (1995 Jun) 53 (1-6) 583-94.
Journal code: 9015483. ISSN: 0960-0760.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19950921
Last Updated on STN: 19950921

linker is a rapidly moving, highly flexible peptide with a random coil-like structure. In X-ray crystallographic investigations of three different sFvs, linkers have also been found to be disordered. Indirect evidence suggests that a monomeric sFv has been crystallized in one case, and dimeric sFvs in the other two.

L47 ANSWER 105 OF 132 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:315562 BIOSIS
DOCUMENT NUMBER: PREV199497328562
TITLE: Identification of the IgG binding ***site*** of the human low affinity receptor for IgG Fc-gamma-RII: Enhancement and ablation of binding by ***site*** -directed ***mutagenesis*** .
AUTHOR(S): Hulett, Mark D.; Witort, Ewa; Brinkworth, Ross I.; McKenzie, Ian F. C.; Hogarth, P. Mark (1)
CORPORATE SOURCE: (1) Austin Res. Inst., Austin Hosp., Studley Rd., Heidelberg, VIC 3084 Australia
SOURCE: Journal of Biological Chemistry, (1994) Vol. 269, No. 21, pp. 15287-15293.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Fc receptor- ***antibody*** interactions are key mechanisms through which ***antibody*** effector functions are mediated. The low affinity receptor for IgG, Fc-gamma-RII, is expressed on most hematopoietic cells, and through the binding of immune complexes mediates a large spectrum of biological responses vital for resistance to infection and the regulation of immunity. In this study the key residues of human Fc-gamma-RII involved in the interaction with IgG1 have been identified. Chimeric receptors composed of extracellular regions of Fc-gamma-RII and the Fc-epsilon-RI alpha chain have been used to localize the IgG1 binding ***site*** of Fc-gamma-RII to an 8-residue stretch in the second extracellular ***domain*** , Asn-154 to Ser-161. ***Site*** -directed ***mutagenesis*** of this region revealed that substitution of Ile-155 or Gly-156 with alanine ablated the binding of human and mouse IgG1, whereas replacement of ***Leu*** -159, ***Phe*** -160, or Ser-161 with alanine enhanced binding. Molecular modeling has been used to generate a putative 3-dimensional model structure of the second extracellular ***domain*** of Fc-gamma-RII, suggesting that the binding ***site*** lies in an exposed loop region at the ***interface*** of domains 1 and 2.

L47 ANSWER 106 OF 132 MEDLINE
ACCESSION NUMBER: 95083639 MEDLINE
DOCUMENT NUMBER: 95083639 PubMed ID: 7991583
TITLE: Rapid humanization of the Fv of monoclonal ***antibody*** B3 by using framework exchange of the ***recombinant*** immunotoxin B3(Fv)-PE38.
AUTHOR: Benhar I; Padlan E A; Jung S H; Lee B; Pastan I
CORPORATE SOURCE: Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Dec 6) 91 (25) 12051-5.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950124
Last Updated on STN: 20020420
Entered Medline: 19950111
AB B3(Fv)-PE38 is a ***recombinant*** single-chain immunotoxin in which the Fv region of carcinoma-specific ***antibody*** B3 is fused to a truncated form of Pseudomonas exotoxin (PE). The efficacy of monoclonal ***antibody*** B3 and B3 immunotoxins in cancer therapy and diagnosis may be limited by the human anti-mouse response. Here we describe the

humanization of the Fv of B3(Fv)-PE38 by "framework exchange." The variable domains of the ***heavy*** (VH) and ***light*** (VL) chains were aligned with their best human homologs to identify framework residues that differ. Initially, 11 framework residues in VH and six in VL were changed by site-specific mutagenesis to human residues and introduced simultaneously into a preassembled single-chain Fv expression cassette. Six VH and five VL residues that differ were not changed because they were buried, in the interdomain ***interface*** , or previously found to result in decreased affinity when mutated. This basic design resulted in some 20-fold loss of activity. Changing VL residues at the interdomain interfacial position 100 and at the buried position 104 to the human sequence increased the activity 8-fold. Changing VH residue at position 82b from the human sequence back to that of the mouse restored the activity 2- to 3-fold to the full binding and cytotoxic activity of the mouse sequence. Humanized B3(Fv)-PE38 lost immunogenic epitopes recognized by sera from monkeys that had been immunized with B3(Fv)-PE38.

L47 ANSWER 107 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94169916 EMBASE
DOCUMENT NUMBER: 1994169916
TITLE: Pressure effects and thermal stability of myosin rods and rod minifilaments: Fluorescence and circular dichroism studies.
AUTHOR: King L.; Chi Chang Liu; Lee R.-F.
CORPORATE SOURCE: Department of Biochemistry, Chang Gung Medical College, 259 Wen-Hwa One Road,Kwei-San, Tao-Yuan 33332, Taiwan,Province of China
SOURCE: Biochemistry, (1994) 33/18 (5570-5580).
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB In the present study hydrostatic pressure was applied upon both skeletal myosin rod molecules and rod minifilaments to learn more of the intra- and intermolecular interaction behavior of myosin. Applied pressure disassembled the rod minifilaments into individual rod molecules and dissociated each myosin rod molecule into two chains of .alpha.-helix. The dissociation and disassembly profiles of these systems were obtained by measuring their fluorescent anisotropy under pressure. The mid-disassembly pressure of rod minifilaments at 0.4 mg/mL concentration was 430-490 bar. However, dissociation of two helical strands of rod molecules occurred at a much higher pressure, with a mid-disassembly pressure of 1300 bar at this concentration. These results indicate that the intramolecular interactions occurring between two .alpha.-helical chains of a rod molecule are much more stable under pressure than the intermolecular interactions that occur among rod molecules in a minifilament. The regions in the rod molecules involved in filament ***assembly*** were investigated through usage of both the intrinsic fluorescence of ***tryptophan*** residues and the extrinsic fluorescence of 6-acryloyl-2-(dimethylamino)naphthalene (acrylodan) labeled cysteine residues. The blue spectral shifts upon minifilament formation suggest the participation of both ***light*** meromyosin (LMM) and subfragment-2 (S-2) regions of myosin rods in the filament formation. Profiles of thermal unfolding of myosin rod molecules and rod minifilaments were obtained by circular dichroism measurement. The multiple transitions exhibited upon unfolding profiles indicated the presence of more than one structural ***domain*** , each correlating with a cooperative transition. The ***domain*** transitional temperatures were found to be 1-4 .degree.C higher for rods in minifilaments than those for rod molecules in a solution of similar ionic composition, indicating that all structural domains are involved in filament ***assembly*** . Furthermore, the ***domain*** transitional temperatures for rod molecules in a buffer containing 0.6 M NaCl were 6-8 .degree.C higher than those for rod molecules in 5 mM sodium pyrophosphate buffer, suggesting that each structural ***domain*** of a rod molecule becomes stabilized

Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940406
Last Updated on STN: 19940406
Entered Medline: 19940330

AB A human ***heavy*** chain variable domain (VH) was expressed in bacteria for structural analysis by NMR spectroscopy. NMR analysis was initially impossible due to the short transverse proton relaxation time of the VH, probably caused by aggregation through the exposed ***interface*** naturally in contact with the ***light*** chain. The relaxation time was improved to normal values when this ***interface*** was mutated to mimic ***heavy*** chains of camel antibodies naturally devoid of ***light*** chains and through the use of the detergent CHAPS. Assignment of NMR signals will now be possible after isotopic labeling. Implications for the design of VH domains as minimum size immunoreagents are outlined.

L47 ANSWER 112 OF 132 MEDLINE
ACCESSION NUMBER: 94291775 MEDLINE
DOCUMENT NUMBER: 94291775 PubMed ID: 8020600
TITLE: Characterization of type II and type XI collagen synthesis by an immortalized rat chondrocyte cell line (IRC) having a low level of type II collagen mRNA expression.
AUTHOR: Oxford J T; Doege K J; Horton W E Jr; Morris N P
CORPORATE SOURCE: Research Department, Shriners Hospital for Crippled Children, Portland, Oregon 97201.
CONTRACT NUMBER: R29 GM38862 (NIGMS)
SOURCE: EXPERIMENTAL CELL RESEARCH, (1994 Jul) 213 (1) 28-36.
Journal code: 0373226. ISSN: 0014-4827.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940815
Last Updated on STN: 19940815
Entered Medline: 19940801

AB The biosynthesis of type XI and type II collagens was examined using a stable rat chondrocyte cell line established by W. E. Horton et al. (1988, Exp. Cell Res. 178, 457-468.). These cells (IRC; immortalized rat chondrocytes) were created by transformation with a murine retrovirus carrying the v-myc and v-raf oncogenes. They grow in suspension culture as multicellular aggregates and synthesize typical cartilage proteins, aggrecan and link protein. Type II collagen is absent or synthesized at severely reduced levels, as shown by Northern analysis of mRNA. Thus, this cell type represents a unique model in which to study cartilage matrix protein interactions in the absence of type II collagen. A more detailed look at the proteins secreted into the medium by metabolically labeled IRC cells revealed the presence of collagenase-sensitive bands when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The bands were identified as the alpha 1, alpha 2, and alpha 3 chains of heterotrimeric type XI collagen by electrophoretic migration after pepsin digestion, by CNBr peptide mapping, and by immunoprecipitation with antibodies to rat alpha 1(XI). mRNA for all three chains was detected by Northern blot analysis. The data indicate that the low level of alpha 1(II) mRNA previously detected in these cells is translated into pro alpha 3(XI) polypeptide chains which are incorporated into molecules of type XI. Under normal culture conditions, homotrimers of type II collagen were not detected. The carboxyl propeptide ***domain*** of the fibrillar collagens directs ***chain*** selection and molecular ***assembly*** of the trimeric molecules. The sequence of the carboxyl propeptide ***domain*** from pro alpha 3(XI) of IRC cells was found to be identical to this ***domain*** from pro alpha 1(II) of swarm rat chondrosarcoma,

ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930416
Last Updated on STN: 19970203
Entered Medline: 19930401

AB E4TF1 was originally identified as one of the transcription factors responsible for adenovirus E4 gene transcription. It is composed of two subunits, a DNA binding protein with a molecular mass of 60 kDa and a 53-kDa transcription-activating protein. ***Heterodimerization*** of these two subunits is essential for the protein to function as a transcription factor. In this study, we identified a new E4TF1 subunit, designated E4TF1-47, which has no DNA binding activity but can associate with E4TF1-60. We then cloned the cDNAs for each of the E4TF1 subunits. E4TF1 was purified, and the partial amino acid sequence of each subunit was determined. The predicted amino acid sequence of each cDNA clone revealed that E4TF1-60 had an ETS domain, which is a DNA binding domain common to ets-related transcription factors. E4TF1-53 had four tandemly repeated notch-ankyrin motifs. The putative cDNA of E4TF1-47 coded almost the same amino acid sequences as E4TF1-53. Three hundred and thirty-two amino acids of the N termini of E4TF1-47 and -53 were identical except for one amino acid insertion in E4TF1-53, and they differ from each other at the C terminus. These three ***recombinant*** cDNA clones were expressed in Escherichia coli, and the proteins behaved in the same manner as purified proteins in a gel retardation assay. Nucleotide and predicted amino acid sequences were highly homologous to GABP-alpha and -beta, which is further supported by the observation that GABP-specific ***antibody*** can recognize human E4TF1.

L47 ANSWER 120 OF 132 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:339228 BIOSIS
DOCUMENT NUMBER: PREV199396036228
TITLE: Ionic interactions in the coiled-coil ***domain*** of laminin determine the specificity of ***chain*** ***assembly*** .
AUTHOR(S): Beck, Konrad (1); Dixon, Tony W.; Engel, Jurgen; Parry, David A. D.
CORPORATE SOURCE: (1) Shriners Hosp. Crippled Children, 3101 S.W. Sam Jackson Park Road, Portland, OR 97201
SOURCE: Journal of Molecular Biology, (1993) Vol. 231, No. 2, pp. 311-323.
ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Laminins are a family of large (800 to 900 kDa) multidomain glycoproteins specifically found in basement membranes. They consist of one ***heavy*** A ***chain*** and two ***light*** chains B1 and B2, and several tissue-specific laminin isoforms exist. ***Chain*** ***assembly*** is mediated through the formation of a rod-like triple-stranded alpha-helical coiled-coil ***domain*** about 75 nm long. The interacting edges of the chains are mostly formed by hydrophobic residues in positions a and d of an (abcdefg)-n heptad sequence repeat and by a distinct pattern of charged residues in positions e and g. Here, we have analyzed the sequences of known laminin chains in an effort to relate them to interaction potential. Initially, those sequences localized in the long arm were arranged in an optimum heptad-repeating scheme. The interacting edges between chains were then analyzed for interchain hydrophobic and ionic interactions. The short heptad blocks were allowed to shift axially with respect to each other to maximize the number of interactions. The number of hydrophobic interactions was very high and similar for all ***chain*** combinations, but especially so for homodimers. As these were not observed experimentally, it seems that hydrophobic interactions probably represent only a prerequisite for coiled-coil formation. The number of ionic interaction;, however, directly resembles the interaction potential observed in in vitro experiments. In particular, the number of interchain ionic interactions is high for parallel heterodimer configurations of A and B chains, but low for homodimer arrangements. When the laminin isoform chains, rat s-laminin (B1s) and human merosin (Am), are included in the analysis, they show

rather low numbers of mutual interactions but high ionic interaction potentials between them and distinct mouse laminin chains are predicted. For mouse laminin the analysis was extended to a full three-stranded coiled-coil structure. The highest number of interchain ionic interactions occurs for an anti-clockwise ***chain*** arrangement of A fwdarw B1 fwdarw B2 when viewed from the N terminus. None of the laminin chains appears to be designed for the formation of homodimers, although such conformations are frequently found in other alpha-fibrous proteins.

L47 ANSWER 121 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:167456 CAPLUS
DOCUMENT NUMBER: 118:167456
TITLE: Method for making humanized antibodies
INVENTOR(S): Carter, Paul J.; Presta, Leonard G.
PATENT ASSIGNEE(S): Genentech, Inc., USA
SOURCE: PCT Int. Appl., 126 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9222653	A1	19921223	WO 1992-US5126	19920615
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE				
CA 2103059	AA	19921215	CA 1992-2103059	19920615
AU 9222509	A1	19930112	AU 1992-22509	19920615
AU 675916	B2	19970227		
EP 590058	A1	19940406	EP 1992-914220	19920615
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
JP 06508267	T2	19940922	JP 1992-501103	19920615
EP 940468	A1	19990908	EP 1999-105252	19920615
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC				
US 6407213	B1	20020618	US 1993-146206	19931117
US 6054297	A	20000425	US 1995-437642	19950509

PRIORITY APPLN. INFO.: US 1991-715272 A2 19910614
EP 1992-914220 A3 19920615
WO 1992-US5126 A 19920615
US 1992-934373 A1 19920821

AB A method for making a humanized ***antibody*** is claimed. The method comprises detg. a sequence for a consensus human variable and a consensus human framework region (FR) and comparing these sequences to the corresponding sequences in the non-human ***antibody*** . The complementary detg. region (CDR) of the non-human ***antibody*** is substituted for that of the consensus human variable region. Residues in the non-human ***antibody*** FR which are not homologous to those in the corresponding human consensus FR are substituted for the human residues if the residue noncovalently binds antigen directly, if it interacts with a CDR, or if it participates in the VL-VH ***interface*** . The method was used to prep. 8 humanized antibodies to p185HER2 from a mouse monoclonal ***antibody*** to this proto-oncogene product. These humanized antibodies were designed to explore the importance of several FR residues. The chimeric genes for the humanized ***heavy*** and ***light*** chains were transiently expressed in 293 cells, and the affinity of the antibodies for sol. p185HER2 and the anti-proliferative effect on p185HER2-overproducing mammary adenocarcinoma cell line SK-BR-3 were detd. One humanized ***antibody*** bound the sol. p185HER2 3-fold more tightly than the murine ***antibody*** and had comparable anti-proliferative activity. However, there was no simple correlation of binding affinity and anti-proliferative activity since an increased anti-proliferative activity was not always accompanied by an increased affinity.

L47 ANSWER 122 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:35073 CAPLUS

protein domains directly leads to a boost of avidity, and it allows the construction of ***bispecific*** ***antibody*** fragments in functional form in E. coli.

L47 ANSWER 124 OF 132 MEDLINE
ACCESSION NUMBER: 93062914 MEDLINE
DOCUMENT NUMBER: 93062914 PubMed ID: 1331778
TITLE: ***Heterodimerization*** among thyroid hormone receptor, retinoic acid receptor, retinoid X receptor, chicken ovalbumin upstream promoter transcription factor, and an endogenous liver protein.
AUTHOR: Berrodin T J; Marks M S; Ozato K; Linney E; Lazar M A
CORPORATE SOURCE: Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia 19104.
CONTRACT NUMBER: 1RO1-DK-43806-01 (NIDDK)
SOURCE: MOLECULAR ENDOCRINOLOGY, (1992 Sep) 6 (9) 1468-78. Journal code: 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19990129
Entered Medline: 19921221
AB Thyroid hormone receptor (TR) binds to DNA as a monomer, homodimer, and heterodimer with nuclear proteins. We have confirmed that the TR can ***heterodimerize*** with retinoid X receptors (RXRs)-alpha and -beta, and have found that another member of the nuclear receptor superfamily, chicken ovalbumin upstream promoter transcription factor (COUP-TF), also formed heterodimers with the TR in the context of binding to a palindromic thyroid hormone-responsive element (TREp). The interaction between COUP-TF and the TR was confirmed using specific antibodies which supershifted the COUP-TF/TR DNA complexes. The complex between the TR and the major TR ***heterodimerization*** partner in liver was unaffected by antibodies to COUP-TF and RXR beta, but was supershifted by an anti-RXR alpha ***antibody*** , indicating that the liver protein is highly related to RXR alpha. Indeed, the TR/RXR and TR/liver protein heterodimers contact the same guanidine residues in TREp. The retinoic acid receptor (RAR) also ***heterodimerized*** with COUP-TF as well as with RXR alpha, RXR beta, and the TR ***heterodimerization*** partner in liver. In contrast to its ability to ***heterodimerize*** with the TR and RAR, we did not detect heterodimers between COUP-TF and either RXR alpha, RXR beta, or the liver nuclear protein in the context of binding to the TREp. These results show that the major TR ***heterodimerization*** partner in liver is highly related to RXR alpha, but that other nuclear receptors such as COUP-TF can ***heterodimerize*** with the TR and RAR, suggesting that selective protein-protein interactions may be involved in the tissue and target gene specificities of hormone action.

L47 ANSWER 125 OF 132 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92298286 EMBASE
DOCUMENT NUMBER: 1992298286
TITLE: Expression and secretion of an ***assembled*** tetrameric CH2-deleted ***antibody*** in E. coli.
AUTHOR: Lo K.-M.; Roy A.; Foley S.F.; Coll J.T.; Gillies S.D.
CORPORATE SOURCE: Dana-Farber Cancer Institute, 840 Mayer, 44 Binney Street,Boston, MA 02115, United States
SOURCE: Human Antibodies and Hybridomas, (1992) 3/3 (123-128). ISSN: 0956-960X CODEN: HANHEX
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
016 Cancer
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English

SUMMARY LANGUAGE: English

AB We have expressed in E. coli a functional ***assembled*** ***antibody*** variant that is secreted into the media. The ***antibody*** variant is a CH2-deleted chimeric ***antibody*** 14.18, which was previously shown to be a potentially useful reagent for radioimmunodetection of human tumors. The bacterial expression vector contains a dicistronic unit comprised of a L- ***chain*** cDNA and a CH2-deleted H- ***chain*** cDNA. For translocation across the bacterial membranes, we have replaced the natural signal peptides of the H and L chains with the signal peptide of the bacterial protein pectate lyase B. When expressed in the JM105 host under the control of the ***trp*** -lac promoter, the products were secreted into the M9 growth media to about 350 .mu.g/L. The secreted ***antibody*** , which can be readily purified from the media without any denaturation or renaturation steps, retains antigen-binding activity. SDS-PAGE and nondenaturing size exclusion high-pressure liquid chromatography showed that it is a mixture of ***assembled*** HL and fully ***assembled*** H2L2. In H2L2, inter-H ***chain*** disulfide bonds are not formed, and the two HL half-molecules are likely held together by the trans interaction between the two CH3 domains.

L47 ANSWER 126 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:79875 CAPLUS
DOCUMENT NUMBER: 116:79875
TITLE: Process and apparatus for separation by carrier-mediated transport
INVENTOR(S): Cohen, Charles M.; Dishman, Robert A.; Huston, James S.; Bratzler, Robert L.; Dodds, David R.; Zepp, Charles M.
PATENT ASSIGNEE(S): Creative Biomolecules, Inc., USA; Sepracor, Inc.
SOURCE: PCT Int. Appl., 107 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9112072	A1	19910822	WO 1991-US627	19910130
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
US 5167824	A	19921201	US 1990-479935	19900214
AU 9172491	A1	19910903	AU 1991-72491	19910130
AU 637884	B2	19930610		
EP 516686	A1	19921209	EP 1991-904736	19910130
EP 516686	B1	19960313		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
JP 05504094	T2	19930701	JP 1991-504476	19910130
AT 135257	E	19960315	AT 1991-904736	19910130

PRIORITY APPLN. INFO.: US 1990-479935 19900214
WO 1991-US627 19910130

AB Disclosed are processes and app. for sepg. a desired solute, such as an optically active isomer, from a complex mixt. using carrier-facilitated transport in an immobilized liq. membrane or carrier-facilitated solvent extn. The carrier is a binding protein selected and/or engineered to immunochem. reversibly bind to the solute and to have a significant soly. in the extg. solvent or immobilized liq. membrane. The app. comprises (a) a 1st membrane; (b) a hydrophilic liq. phase in contact with the membrane; (c) means for passing a hydrophobic feed soln. into contact with the membrane ***interface*** , the feed soln. contg. the desired solute in a solvent immiscible with the hydrophilic phase; and (d) a binding protein dissolved in the hydrophilic phase for immunochem. binding the solute at the membrane ***interface*** . Various app. and process embodiments are described and diagrammed. A genetically-engineered single-chain fusion protein, comprising the ***heavy*** - and ***light*** -chain variable region binding sites of a monoclonal ***antibody*** to

digoxin, was prepd. and used to ext. oleandrin in a supported liq. membrane process. Resoln. of naproxen is also described.

L47 ANSWER 127 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:625446 CAPLUS
DOCUMENT NUMBER: 115:225446
TITLE: CD3-specific humanized antibodies
INVENTOR(S): Jolliffe, Linda Kay; Zivin, Robert Allan; Adair, John Robert; Athwal, Diljeet Singh
PATENT ASSIGNEE(S): Celltech Ltd., UK
SOURCE: PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9109968	A1	19910711	WO 1990-GB2018	19901221
W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, GR, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, RO, SD, SE, SU, US				
RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG				
CA 2046904	AA	19910622	CA 1990-2046904	19901221
CA 2050479	AA	19910622	CA 1990-2050479	19901221
CA 2050479	C	19970325		
AU 9170330	A1	19910724	AU 1991-70330	19901221
AU 649645	B2	19940602		
EP 460171	A1	19911211	EP 1991-901559	19901221
EP 460171	B1	19950628		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
HU 58372	A2	19920228	HU 1991-2734	19901221
HU 215383	B	20000328		
HU 58824	A2	19920330	HU 1991-2752	19901221
HU 60786	A2	19921028	HU 1991-2751	19901221
JP 04506458	T2	19921112	JP 1991-501865	19901221
JP 3242913	B2	20011225		
GB 2268744	A1	19940119	GB 1993-18911	19901221
GB 2268744	B2	19940511		
GB 2268745	A1	19940119	GB 1993-18912	19901221
GB 2268745	B2	19940511		
EP 620276	A1	19941019	EP 1994-104042	19901221
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
EP 626390	A1	19941130	EP 1994-202090	19901221
EP 626390	B1	20011114		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
ES 2074701	T3	19950916	ES 1991-901559	19901221
AT 129017	E	19951015	AT 1991-901433	19901221
ES 2079638	T3	19960116	ES 1991-901433	19901221
AT 159299	E	19971115	AT 1991-901835	19901221
ES 2112270	T3	19980401	ES 1991-901835	19901221
RO 114232	B1	19990226	RO 1990-148281	19901221
JP 11243955	A2	19990914	JP 1997-353861	19901221
AT 208794	E	20011115	AT 1994-202090	19901221
ES 2165864	T3	20020401	ES 1994-202090	19901221
CA 2037607	AA	19920907	CA 1991-2037607	19910306
CA 2129219	C	19981222	CA 1991-2129219	19910306
GB 2246781	A1	19920212	GB 1991-17611	19910815
GB 2246781	B2	19940511		
NO 9103229	A	19911021	NO 1991-3229	19910819
US 5929212	A	19990727	US 1993-116247	19930903
AU 9464612	A1	19941222	AU 1994-64612	19940608
AU 664801	B2	19951130		
US 5859205	A	19990112	US 1994-303569	19940907
NO 9805467	A	19911021	NO 1998-5467	19981123

PRIORITY APPLN. INFO.: GB 1989-28874 A 19891221

EP 1991-901433 A3 19901221
JP 1991-501864 A3 19901221
WO 1990-GB2018 A 19901221
CA 1991-2037607 A3 19910306
GB 1991-17611 A3 19910815
GB 1991-17612 A3 19910815
US 1991-743329 B1 19910917

AB ***Recombinant*** CD3-specific antibodies that contain const. regions from one species (e.g. human), complementary-detg. regions (CDRs) from another species (e.g. mouse), and other alterations outside of the CDRs to enhance affinity are prepd. Several non-CDR residues were identified which contribute to antigen binding. These were surface residues near the CDRs, residues near the DCRs which contribute to stable side-chain packing, residues at the variable domain ***interface*** between ***heavy*** and ***light*** chains, and residues at the variable-const. region ***interface*** . One such ***recombinant*** ***antibody*** , contg. mouse CDRs, 11 mouse framework amino acids in the ***heavy*** chain and 2 mouse framework amino acids in the ***light*** chain had binding characteristics very similar to that of the donor Ig OKT3. These antibodies can be used in diagnosis and therapy.

L47 ANSWER 128 OF 132 MEDLINE
ACCESSION NUMBER: 91045952 MEDLINE
DOCUMENT NUMBER: 91045952 PubMed ID: 2122454
TITLE: A single amino acid substitution in the variable region of the ***light*** ***chain*** specifically blocks immunoglobulin secretion.
AUTHOR: Dul J L; Argon Y
CORPORATE SOURCE: Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1990 Oct) 87 (20) 8135-9.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199012
ENTRY DATE: Entered STN: 19910208
Last Updated on STN: 19910208
Entered Medline: 19901204

AB Although immunoglobulin ***light*** chains are usually secreted in association with ***heavy*** chains, free ***light*** chains can be secreted by lymphocytes. To identify the structural features of ***light*** chains that are essential for their secretion, we mutated a conserved sequence in the variable ***domain*** of a lambda I ***light*** ***chain*** . The effects of the mutations on secretion were assayed by transient expression in COS-1 cells. One mutant (AV60), which replaced Ala-60 with Val, was secreted as efficiently as wild-type lambda I by transfected COS-1 cells. This result was not surprising because secreted lambda II chains contain valine in this position. However, a second lambda I mutant (AV60FS62), which replaced ***Phe*** -62 with Ser as well as Ala-60 with Val, was not secreted. This mutant was arrested in the endoplasmic reticulum, as judged by immunofluorescence and by its association with a lumenal endoplasmic reticulum protein, immunoglobulin ***heavy*** ***chain*** binding protein (BiP). The defect in secretion was not due to gross misfolding of the lambda I ***chain*** , since cells cotransfected with AV60FS62 and an immunoglobulin ***heavy*** ***chain*** gene produced functional antigen-binding antibodies. These ***assembled*** IgM molecules were still not secreted. Hence, the replacement of ***Phe*** -62 with Ser specifically affects a determinant on the lambda I ***light*** ***chain*** that is necessary for the intracellular transport of this molecule.

L47 ANSWER 129 OF 132 MEDLINE
ACCESSION NUMBER: 90347830 MEDLINE

DOCUMENT NUMBER: 90347830 PubMed ID: 2200886
TITLE: Function of Semliki Forest virus E3 peptide in virus assembly: replacement of E3 with an artificial signal peptide abolishes spike ***heterodimerization*** and surface expression of E1.
AUTHOR: Lobigs M; Zhao H X; Garoff H
CORPORATE SOURCE: Department of Molecular Biology, Karolinska Institute, Huddinge, Sweden.
SOURCE: JOURNAL OF VIROLOGY, (1990 Sep) 64 (9) 4346-55. Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901026
Last Updated on STN: 19990129
Entered Medline: 19900914

AB The Semliki Forest virus spike glycoproteins E1 and p62 form a heterodimeric complex in the endoplasmic reticulum (ER) and are transported as such to the cell surface. In the mature virus particle, the heterodimeric association of E1 and E2 (the cleavage product of p62) is maintained, but as a more labile and acid-sensitive oligomer than the E1-p62 complex. The E3 peptide forms the N-terminal part of the p62 precursor and carries the signal for the translocation of p62 into the lumen of the ER. The question of whether E3 is also important in the formation and stabilization of the E1-p62 heterodimer has been addressed here with the aid of an E3 deletion mutant cDNA. In this construct, the entire E3 was replaced with a cleavable, artificial signal sequence which preserved the membrane topology of an authentic E2. The E3 deletion, when expressed via a ***recombinant*** vaccinia virus, abolished ***heterodimerization*** of the spike proteins. It also resulted in the complete retention of E1 in the ER and almost total inhibition of E2 transport to the plasma membrane. The oligomerization and transport defect of E1 expressed from the E3 deletion mutant could be complemented with a wild-type p62 provided from a separate coding unit in double infections. These results point to a central role of E3 in complex formation and transport of the viral structural components to the site of budding. In conjunction with earlier work (M. Lobigs and H. Garoff, J. Virol. 64:1233-1240, 1990; J. Wahlberg, W. A. M. Boere, and H. Garoff, J. Virol. 63:4991-4997, 1989), the data support a model of spike protein oligomerization control of Semliki Forest virus assembly and disassembly which may be mediated by the presence of E3 in the uncleaved p62 precursor and release of E3 after cleavage.

L47 ANSWER 130 OF 132 MEDLINE
ACCESSION NUMBER: 91297182 MEDLINE
DOCUMENT NUMBER: 91297182 PubMed ID: 1712503
TITLE: Conformational change in the N-terminal ***domain*** of the Escherichia coli ***tryptophan*** synthase beta 2 subunit induced by its interactions with monoclonal antibodies.
AUTHOR: Blond-Elguindi S; Goldberg M E
CORPORATE SOURCE: Unite de Biochimie Cellulaire, CNRS URA 1129, Institut Pasteur, Paris.
SOURCE: RESEARCH IN IMMUNOLOGY, (1990 Nov-Dec) 141 (9) 879-92. Journal code: 8907467. ISSN: 0923-2494.
PUB. COUNTRY: France
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 19910901
Last Updated on STN: 19970203
Entered Medline: 19910809

AB A fluorescence energy transfer signal was used to follow conformational changes occurring in two types of protein-protein complexes. The first

complex studied was the native-like beta 2 subunit of Escherichia coli ***tryptophan*** synthase reconstituted by reassembly of the N- and C-terminal proteolytic domains of the beta ***chain*** . The other complexes were formed by the association of the N-terminal fragment (F1) with a monoclonal ***antibody*** that recognizes the native dimeric protein; four such complexes, obtained with different antibodies that recognize four distinct antigenic sites on native beta 2, were investigated. It was shown that a structural readjustment, which the isolated F1 ***domain*** was unable to undergo alone, was imposed upon F1 by interdomain interactions. Furthermore, with three of the four antibodies studied, the same conformational change in F1 also occurred after formation of the F1- ***antibody*** complex. These results demonstrate that, through an "induced fit mechanism", antigen- ***antibody*** stereospecific ***assembly*** can force the polypeptide ***chain*** to adopt a structure more closely resembling the conformation it has in the native protein.

L47 ANSWER 131 OF 132 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:570316 CAPLUS
DOCUMENT NUMBER: 113:170316
TITLE: Recombinant antibodies to Campath-1 antigen, containing foreign complementarity determining region(s), and their use in immunosuppression and cancer therapy
INVENTOR(S): Waldmann, Herman; Clark, Michael Ronald; Winter, Gregory Paul; Riechmann, Lutz
PATENT ASSIGNEE(S): Medical Research Council, UK
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 8907452	A1	19890824	WO 1989-GB113	19890210
W: AU, DK, JP, US				
EP 328404	A1	19890816	EP 1989-301291	19890210
EP 328404	B1	19930929		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AU 8930626	A1	19890906	AU 1989-30626	19890210
AU 618989	B2	19920116		
GB 2216126	A1	19891004	GB 1989-2978	19890210
GB 2216126	B2	19920603		
JP 02503514	T2	19901025	JP 1989-502205	19890210
JP 3121823	B2	20010109		
ZA 8901069	A	19901031	ZA 1989-1069	19890210
AT 95068	E	19931015	AT 1989-301291	19890210
ES 2059719	T3	19941116	ES 1989-301291	19890210
CA 1339198	A1	19970805	CA 1989-590704	19890210
JP 11228600	A2	19990824	JP 1998-329954	19890210
DK 8905021	A	19891012	DK 1989-5021	19891010
US 5846534	A	19981208	US 1994-235705	19940429

PRIORITY APPLN. INFO.: GB 1988-3228 A 19880212
GB 1988-4464 A 19880225
EP 1989-301291 A 19890210
JP 1989-502205 A3 19890210
WO 1989-GB113 A 19890210
US 1989-424233 B1 19891012
US 1992-921601 B1 19920803
US 1993-99480 B1 19930730

AB Antibodies which effectively bind Campath-1 antigen are produced having .gtoreq.1 complementarity detg. region (CDR) which is foreign with respect to the const. region of the ***antibody*** . The amino acid sequence of the CDRs are specified and are selected from amino acid residues 31-35, 50-65, and 95-102 of the ***heavy*** ***chain*** and residues

24-34, 50-56, and 89-97 of the ***light*** ***chain*** . The ***heavy*** and ***light*** ***chain*** const. domains are selected from antibodies of different species and/or different classes. The antibodies are useful in cancer therapy and in immunosuppression. A chimeric ***antibody*** was constructed having the CDRs of rat IgG2a Campath-1 ***antibody*** YTH 34.5 HL mounted on human ***heavy*** or ***light*** ***chain*** framework regions taken from the NEW protein for the ***heavy*** ***chain*** and from a protein based closely on the human myeloma protein REI for the ***light*** ***chain*** . The resulting reshaped ***heavy*** ***chain*** variable ***domain*** HuVHCAMP was based on the HuVHNP gene with the framework regions of human NEW alternating with the hypervariable regions of rat YTH 34.5 HL. The reshaped ***light*** ***chain*** variable ***domain*** HuVLCAMP had the framework of REI with the C-terminal and 3' noncoding sequence of human Jk-region sequence. The reshaped human ***heavy*** and ***light*** ***chain*** variable domains were ***assembled*** with const. domains in 3 stages. A mutant of the ***antibody*** , HuVHCAMP (Ser 27 to ***Phe*** , Thr 30 to Ser)-HuIGG1, HuVLCAMP-HuIGK (CAMPATH-1H), was used to treat 2 patients having grade 1 IVA nonHodgkins lymphoma in leukemic phase and lymphoplasmacytoid, resp. The remissions achieved show that it is possible to clear large nos. of tumor cells with small amts. of ***antibody*** . No antiglobulin to CAMPATH-1H was detected but some toxic effects were noted.

L47 ANSWER 132 OF 132 MEDLINE
ACCESSION NUMBER: 89352546 MEDLINE
DOCUMENT NUMBER: 89352546 PubMed ID: 2475171
TITLE: On the attribution of binding energy in antigen-***antibody*** complexes McPC 603, D1.3, and HyHEL-5.
AUTHOR: Novotny J; Bruccoleri R E; Saul F A
CORPORATE SOURCE: Massachusetts General Hospital, Boston 02114.
SOURCE: BIOCHEMISTRY, (1989 May 30) 28 (11) 4735-49.
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AB Using X-ray coordinates of antigen- ***antibody*** complexes McPC 603, D1.3, and HyHEL-5, we made semiquantitative estimates of Gibbs free energy changes (delta G) accompanying noncovalent complex formation of the McPC 603 Fv fragment with phosphocholine and the D1.3 or HyHEL-5 Fv fragments with hen egg white lysozyme. Our empirical delta G function, which implicitly incorporates solvent effects, has the following components: hydrophobic force, solvent-modified electrostatics, changes in side-chain conformational entropy, translational/overall rotational entropy changes, and the dilutional (cratic) entropy term. The calculated delta G ranges matched the experimentally determined delta G of McPC 603 and D1.3 complexes and overestimated it (i.e., gave a more negative value) in the case of HyHEL-5. Relative delta G contributions of selected ***antibody*** residues, calculated for HyHEL-5 complexes, agreed with those determined independently in ***site*** -directed ***mutagenesis*** experiments. Analysis of delta G attribution in all three complexes indicated that only a small number of amino acids probably contribute actively to binding energetics. These form a subset of the total antigen- ***antibody*** contact surface. In the antibodies, the bottom part of the antigen binding ***cavity*** dominated the energetics of binding whereas in lysozyme, the energetically most important residues defined small (2.5-3 nm2) "energetic" epitopes. Thus, a concept of protein antigenicity emerges that involves the active, attractive contributions mediated by the energetic antigenic epitopes and the passive surface complementarity contributed by the surrounding contact area. The D1.3 energetic epitope of lysozyme involved Gly 22, Gly 117, and

Gln 121; the HyHEL-5 epitope consisted of ***Arg*** 45 and ***Arg*** 68. These are also the essential antigenic residues determined experimentally. The above positions belong to the most protruding parts of the lysozyme surface, and their backbones are not exceptionally flexible. Least-squares analysis of six different ***antibody*** binding regions indicated that the geometry of the VH-VL ***interface*** beta-barrel is well conserved, giving no indication of significant changes in ***domain*** - ***domain*** contacts upon complex formation.